

On the histopathologic lesions of the bone-marrow in immunisation for agglutinin production

by

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(With plates 17—22.)

The research work carried out during the last few years tends to indicate the blood forming organs, bone-marrow, spleen, and lymphatic glands, as the seat of production of antibodies.

Applications of X rays, benzol, thorium X, etc., produce lesions in the blood-forming organs of animals undergoing immunisation and modify the graphics relating to the production of various antibodies.

On the other hand the concentration of antibodies in the serum is considerably increased by substances that stimulate the blood-forming organs, such as arsacetin and salvarsan, independen-

tly of a new injection of antigen. Fragments of spleen and bone-marrow cultivated outside the body, are also able to produce antibodies. Each of these organs produces certain kinds of antibodies; thus the agglutinins are produced chiefly by the bone-marrow, which is not however the hemolysin-producing organ.

It is as yet impossible to ascertain the function of the liver and ductless glands in the production of antibodies, as research work on the subject has yielded such contractory results. It seems likely that the ductless glands (specially the thyroid and the parathyroid) do not actually produce antibodies

but merely influence and control the concentration of these antibodies in the serum.

Most of the literature furnishes scant information on histopathologic lesions of the blood-forming organs, dealing merely with the physiologic side of the question, the subject considered being the antibody producing capacity of the bone-marrow.

The complexity of the origin of antibodies amply justifies an interest in histopathologic research which may furnish a solid basis for discussion.

There is an abundant literature on the subject of immunity but almost none of it deals with this side of the question.

GUERRINI (1903) immunised rats with broth cultures of *Bacillus murisepticus* and found histological lesions in lungs, heart, kidneys and nervous system, a few hours after the injection of the immunising substances. There was nothing characteristic about these, they were quite similar to the ones produced in all intoxications; the lesions of the adrenals, liver, spleen and bone-marrow, however, showed other alterations besides; in adrenals and liver nuclear turgescence and chromatin-disaggregation; in spleen and bone-marrow: numerous macrocytes with polymorphic nuclei.

GAY and RUSK (1913) immunised rabbits by repeated intra-venous injections of washed guinea-pig corpuscles and inoculated again fresh washed corpuscles after an interval of two or more weeks. They made a careful histopathological examination of subjects killed successively, 1, 4 and 24 hours, 4 and 6 days after inoculation and attempted to show evidences of functional activity of certain cells which might be supposed to have formed the antibodies. The only alteration found was the striking increase of glycogen in the liver of the subject killed 24 hours after injection. They themselves are doubtful as to the interpretation which should be given to this.

METALNIKOW and GASCHEN (1922) studied in Intertebrates, (Larvæ of *Galeria*), the processes of immunity principally the changes in the blood. At first there is a reaction of the various leucocytes and phagocytes and after that a phagocytic reaction; in the third place a leucolysis and phagolysis with the freeing of intracellular ferments and antibodies. Shortly afterwards there is a reaction of the spherular cells, which seem to play an important part in immunity. Finally there is a formation of giant-cells and of capsules. The above-mentioned authors think that immunity is the result of a very complex reaction of the cells of the organism and that these reactions are specific for each microbe injected.

SESTINI (1922) observed a thyroid hyperfunction in immunised guinea-pigs (*B. typhi*) which takes the form not only of increase of lipoids and fuchsinophile granules and modification of colloid substances but also of changes in the cells of the vesicles and intravesicular thyroid epithelium, which correspond really to a state of telangectoid hyperplastic struma.

The publications of FOA (1889), DOMINICI (1900), LENGEMANN (1901), MUIR (1901) LONGCOPE (1915), EVANS (1916), etc. on the alterations in these organs in different infections and the production of leucocytes in leucocytosis furnish indirect information on the histopathologic lesions in immunity.

History.

The hypothesis on the origin of antibodies, deduced from the leading opinion of different research-workers can be thus expressed:

1°) All the cells in the body take part in the formation of antibodies; the cellular protoplasm is the seat of the production of antibodies; as an immediate consequence we have the opinion that many tissues can produce antibodies locally.

The primary hypothesis of EHRLICH, according to which all the cells in the body would take a part in the formation of antibodies, is sufficiently known. The local production of antibodies was put up by the first research-workers (ROEMER, VON DUNGERN, WASSERMANN and CITRON). Subsequent research cast doubts on this opinion and indicated that the contrary was the case. HEKTOEN (1911) carried out experiments, the result of which do not favour the local production of specific antibodies, in dogs injected with rat and goat corpuscles, at any rate in the tissue of the pleura, the cellular subcutaneous tissue and that to be found in the anterior chamber of the eyeball. Injection of red corpuscles of rat or goat in the anterior chamber of the eyeball in dogs is followed by the appearance of specific antibodies in the blood and frequently in the aqueous humor. Antibody concentration is greater in the aqueous humor of the injected eye but is decidedly less in both eyes than in the blood; antibodies do not appear first in the aqueous humor but in blood. Injection of rabbit or goat corpuscles in the pleural cavity of dogs is followed by the appearance of specific antibodies in the blood and in the pleural exudates, having been produced by the injection of aleuronate. The concentration in the pleural exudates is not greater than in the blood.

and often is less, the concentration in the blood being in this case slightly lesser than after intravenous injection of the same quantity of antigen. There is no difference in the proportion between the richness in antigen of the blood and the pleural exsudates in dogs, injected with antigen in the pleural cavity, and dogs, injected intravenously. Dogs inoculated subcutaneously with blood corpuscles of goat or rat on the fore leg, do not show a lesser production of antibodies than others on amputation during first phases of antibody-production.

Besides, if antibodies were produced locally at the site of injection of antigen in subcutaneous cellular tissue, it would be reasonable to expect subcutaneous injection of antigen in many places to increase the production of antibodies, which has not been found to be the case.

ZINSSER (1918) seems to be inclined to admit that the formation of antibodies is not the function of special organs but that many of the cells of the body are able to take part in the process; this opinion is founded principally upon the experiments on local immunisation of WASSERMANN, CITRON, and ROEMER, which, as has been seen, were afterwards contested.

Of late OSHIKAWA (1921) managed to graft the skin of actively immunised rabbits on normal rabbits and to ascertain the formation of antibodies in the latter. His protocols, however indicate this antibody-formation to be very small, thus in two cases the titre of the agglutinating serum of the rabbits, on which the grafting was carried out, did not exceed 1/10, although in one case the titre of the rabbit that furnished the skin fragments was equal to 1:640 (immunised with *B. paratyphi B*); in other experiments the serum of the rabbit, on which the skin fragments were grafted, attained the titre of 1:160 at the end of 9 days (immunisation with *B. proteus*). We find the concentration of agglutinin in the serum of the rabbits on which the grafting was carried out very slight to permit categorical conclusions; it must be remembered that the blood forming organs of the rabbit, specially the bone-marrow, are very sensitive to pathologic lesions; it would be quite plausible to suppose that the grafting of the skin alone had influenced the antibody contents of the blood; the operation might have had a similar influence to the injection of peptone or of an irritating substance into the bone-marrow.

2°) The antibodies are produced specially by some of the cells of the organism, principally the leucocytes of the blood.

METCHNIKOFF (1897) had the idea that the bacterioid substances of the serum might be produced by the leucocytes.

BORDET (1895), following this theory, ascertained that the serum had a greater preventive value than the plasma (which contains few leucocytes) and concluded that the leucocytes play an important part in the production of protective substances.

GRUEBER (1897) suggested that the polymorphonu-

clear leucocytes form the agglutinin. This was found to be a mistake by the experiments of ACHARD and BENSUAUDE, WIDAL and SICARD, PATSCH and KRAUS and SCHIFFMANN.

In the case of antitoxins, it was thought that the cells specially attacked by the toxin were the ones that produced the antibody; an instance of this are the ideas of WASSERMANN and TAKAKI on the formation of tetanus antitoxin by the nervous cells. This was immediately found to be erroneous by METCHNIKOFF and MARIE.

V. DUNGERN (1902) finds that the blood-cells take part in the formation of precipitins.

KRAUS and LEVADITI (1904), dosing precipitins in the organs of immunised animals, found that, of all the organs studied, only the epiploon formed highly-precipitating extracts; as the epiploon harbours an accumulation of leucocytes, they concluded that these were chiefly responsible for the antibodies,

KRAUS and SCHIFFMANN (1906) found that rabbits having undergone splenectomy produce precipitating sera, as well as normal ones; they think that precipitins are not formed in the organs but in the vascular system, since, with the exception of the epiploon, no organ possesses precipitins before the serum.

The same authors (1906) ascertained that agglutinins can appear in considerable proportions in the serum without existing at the same time in the extracts of organs; when found in them, it is perceptibly less concentrated than in the blood; the bone marrow has a greater quantity of agglutinins than the spleen and lymphatic glands.

STENSTRÖM (1911), inoculating polymorphonuclear leucocytes together with the antigen (*B. typhi*), observed a decrease in the formation of agglutinins; in consideration of this he is of the opinion that the leucocytes either produce agglutinins or contribute indirectly to their production.

FONSECA (1912) thinks that the leucocytes play the most important part in the formation of antibodies; his hypothesis is based on the fact that "organs attacked by infections that confer immunity in a greater or lesser degree, generally show, on specific counting, an increased number of lymphocytes".

BACHMANN (1918-1919) showed that the leucocytes of immunised animals acquire an important specific property which protects guinea pigs when injected together with *B. typhi* in the peritoneum; the author extracted from the leucocytes the products that ensure this protection.

The results of LEVADITI and BANU's experiments (1920) do not speak in favour of the local formation of agglutinins in the subcutaneous cellular tissue; thus the inflammatory process and oedema, which constitute the local lesion when one injects an emulsion of *B. typhi* with gelatin and colloidal mercury, do not influence favourably but on the contrary hinder the formation of agglutinating antibodies.

TISCORNIA (1921) ascertained that leucocyte extracts of guinea-pigs, immunised against *B. typhi*, when

inoculated in the peritoneum together with a minimum lethal dose, showed quite definite protective properties. He concludes from his experiments that in a phase of immunisation the leucocytes, and specially the neutrophils play a part in the production or modification of special cellular substances; the demonstration of these substances is easy when they are freed suddenly and violently from the leucocytes, but in the circulating blood they are probably secreted in a certain quantity. One may deduct that the production of these special immunising leucocytic substances and their appearance in the circulation being more prompt and intense, is a result of the leucolysis following every leucocytosis.

METALNIKOW and GASCHEN (1922) attribute an important part in the formation of antibodies in invertebrates (*Galleria* larvae) to various cells of the blood of these insects.

ROBERTSON and ROUS (1922) assert the existence of intercellular agglutinins in the red blood-corpuscles of the rabbit, and declare them to be easily demonstrable in the watery extracts of dried corpuscles.

4^o) The antibodies are formed by hematopoietic organs:

PFEIFFER and MARX (1898) titrating simultaneously bactericid antibodies in serum and leucocyte-extracts, demonstrated that the leucocyte-extracts showed no excess of antibodies as compared to the serum and ascertained besides a decided accumulation of antibodies in certain organs of the rabbit (spleen, bone-marrow, lymphatic glands, and to a lesser extent, the lungs) during the first days of immunisation. The spleen showed, already on the second day, a perceptible quantity of protective substances against cholera, although the blood showed no sign of a specific alteration. They thought the hematopoietic organs to be the point of origin of the antibodies, and that any excess of antibodies in these organs would represent an excess of production not accompanied by an equally rapid elimination into the blood.

DEUTSCH (1899) ascertained that splenectomy preceding an immunising injection does not prevent the formation of agglutinins; carried out 3-5 days after the injection it prevents quite clearly the formation of antibodies.

V. EMDEN (1899) immunising rabbits with *B. aerogenes* and searching for agglutinins in the blood and various organs verified that sometimes spleen extracts have a higher agglutinating titre than the blood; agglutinin-formation is hindered by splenectomy, but even so continues; he therefore admits that besides the spleen other organs, specially lymphoid organs are able to elaborate agglutinins.

WASSERMANN (1899) studied the action of sera and extracts of different organs of rabbits immunised with virulent pneumococci on the course of experimental pneumococcus infection. The extracts of bone-marrow showed greater quantities of antibodies than any other organ, and, in the first stages of immunisation the pro-

TECTIVE power of bone-marrow and of other blood forming organs exceeded that of the serum. He thinks that the bone-marrow is the seat of the production of antibodies and that the lymphatic glands, the thymus and spleen are simply reservoirs.

JATTA (1900) ascertained that the agglutinating titre (*B. typhi*) of spleen extracts, between second and fourth day of immunisation, is considerably superior to that of the blood, equal to it on the fourth day, and considerably inferior to it on the eighth day.

HEKTOEN (1909-1910) pointing out the irregularity of the graphics of the production of different antibodies in the same animal suggested that they are distinct substances, the production of which depends on a similar but not identical mechanism.

LIPPMANN (1911) after immunising animals with repeated inoculations of killed cultures of *B. typhi*, left them for 4 months during which the agglutinating titre decreased gradually to a constant mean figure; then injected them with 0,1 gr of arsacetin, a substance whose stimulating influence on the blood forming organs is well known and applied in the treatment of anemia. He ascertained a rapid rise in the titre of the agglutinins which attained its maximum at the end of 6-9 days.

HEKTOEN (1916) verified a reduction in the formation of antibodies in animals intoxicated with benzol together with severe lesions of the bone-marrow, leucopenia and other lesions characteristic of benzol-intoxication. He found also a reduction of the phagocytic properties of the leucocytes. In dogs, small doses of benzol which produce leucocytosis, increase the production of antioxygens.

Benzol acts on the elements which elaborate antibodies and the leucocytogenic centres take part in this elaboration. This is demonstrated in rabbits by the reduction in number of the leucocytes and in quantity of the antibodies, which does not take place when the benzol is given at the time when the production of antibodies is nearly at its height; in dogs by the increased formation of lysins which is accompanied by leucocytosis.

CARREL and INGEBRIGSTEN (1912) ascertained that fragments of bone-marrow and of lymphatic glands cultivated outside the organism are able to produce antibodies (hemolysins).

LUDKE (1912) ascertained the production of agglutinins and hemolysins in bone-marrow and pieces of spleen taken from guinea-pigs and rabbits 24, 48 and 60 hours after the intravenous injection of killed cultures of *B. typhi* and *B. dysenteriae* and kept aseptically in saline solution, solution of RINGER, and normal rabbit and guinea-pig serum at 37°-40° C. At the 5th day of cultivation the spleen emulsion agglutinated up to 1:160 and bone-marrow 1:320.

Inoculating directly in the bone-marrow and killing the animal at the end of 36-48 hours, amputating the femur and cultivating it in the above-mentioned culture media, he was also able to verify the existence of bactericid antibodies and agglutinins in the emulsion of bone-marrow.

TSURUMI and KOHDA (1913) came to the conclu-

sion that the spleen is the most important site of production of complement fixing antibodies and that it already contains them 20 hours after immunisation; the production in bone-marrow and lymphatic glands is not as pronounced as in the spleen, and the quantity found later is also less.

LIPPMANN (1914) studied the influence of thorium X, and arsenical compounds on the graphics of antibody production. In animals previously immunised and allowed a month's rest, he found an increase of the agglutinins (*B. typhi*) in the serum surpassing the previously attained maximum after an application of thorium X (about 1 1/2 electrostatic units per kilogramm of weight). This he attributes to a stimulating influence of thorium on the bone-marrow. Entirely similar researches carried out with antibodies of the nature of amboceptors (hemolysins) gave negative results; neither by the application of thorium X, nor by the application of salvarsan, which has also a distinctly stimulating action on bone-marrow, did he obtain any modification in the graphics of hemolysin production. Animals (mice) injected with bone-marrow-stimulating substances (salvarsan and thorium X) resist an infection (cultures of pneumococcus) lethal to controls.

SIMONDS and JONES (1916) made researches on the influence of benzol on the production of antibodies; this substance exerts a decidedly noxious action on the blood forming organs, specially on the bone-marrow. They noticed a lowering in the graphics of the production of hemolysins, agglutinins and opsonins as compared with controls; this reduction was most accentuated in the case of hemolysins and least in that of opsonins.

SIMONDS and JONES (1915) investigated the modifications in the production of antibodies, on the one hand in rabbits subjected to the action of X rays, which have a specific destructive action for lymphadenoid tissue, on the other hand on animals treated with benzol, which exerts a specific destructive action on the bone-marrow. The action of X rays is not as specific as was supposed; since HEINKE demonstrated that it also produced lesions in the bone-marrow, a fact which ought to be taken into account when reading SIMONDS and JONES results. These are as follows: a) the formation of agglutinins in animals exposed to X-rays is markedly reduced, though not as much as in rabbits inoculated with benzol; b) bacteriolysin-formation does not appear to be much influenced by exposure to x-rays; c) there is no perceptible modification in the contents of the serum in opsonins and of the complement-fixing power in rabbits exposed to X-rays.

HEKTOEN (1918), who stands for the formation of the antibodies by the blood forming organs, ascertained that the exposure of animals to X-rays harmed considerably and sometimes even prevented entirely the formation of antibodies, when carried out at the time of the injecting of antigen, having, on the contrary, no effect when the injection was made at the time when the production of antigens was at its height; a similar resistance was shown by animals inoculated with benzol in the period of active production of antibodies.

Experiments of HEKTOEN (1920) seem to indicate clearly that, after the process of formation of antibodies is well on its way, splenectomy has little or no influence of the contents of the serum in antibodies, although at times, its effect was uncertain and variable; thus in a rabbit after the injection of a big dose of sheep blood, splenectomy has little or no effect on the production of antibodies; on the other hand if it is carried out on the same animal, even many weeks beforehand, it has an influence on the formation of precipitins.

MORESCHI and VOTKY and HOWELL (1920) observed the absence of agglutinin and opsonin formation (HOWELL) in patients of leukaemia in which lesions of the bone-marrow are intense.

An argument in favour of the formation of agglutinins by hæmatopoietic organs and specially by the bone-marrow, consists in the regeneration of the blood provoked in immunised animals, by repeated bleedings and accompanied by an increased production of antibodies. This fact was established by HAHN and LANGER; the authors who reproduced their technic did not confirm their results. It seems, however, that these depend on the opportuneness of the bleeding, for JOTTEN (1920), who was unable to obtain any results with HAHN and LANGER's technic obtained an increase of 40 to 100 times (according to whether the results were read after 2 or after 24 hours) on the titre of the serum in agglutinins (*B. typhi*), by bleeding rabbits of 5 or of 20 cc. from the second day of inoculation.

Intravenous injections of chlorides of manganese, nickel, cobalt and zinc produce a marked and rapid increase in the concentration of agglutinins and diphtheria antitoxin (WALBUM, 1921).

50) Organs other than the blood-forming ones also influence the production of antibodies.

M'GOWAN (1909), studying the lesions of the organs of rabbits inoculated with chicken red-blood corpuscles, noticed an accumulation of these injected corpuscles in the sinusoids of the liver, to a greater extent than in any other organ and lasting much longer; he thought this accumulation in the liver, together with the known phagocytic activity of the hepatic cells and their well-known action on the products of digestion, reinforced the idea that the liver was the seat of the formation of antibodies. These are very unsubstantial facts to support such an assertion.

NOLF and MULLER (1911) are of the opinion that natural cytolytins (normal alexins and amboceptors) have their origin somewhere about the liver. Their principal experiments try to demonstrate the prompt disappearance of natural complement and amboceptors after the suppression of the hepatic circulation, and their persistence after an extreme traumatism, such as the extirpation of all the abdominal organs with the exception of the liver, provided the latter remain physiologically (functionally) intact, as also the possibility of increasing the

alexia and sensitising power of the blood by making it circulate in live isolated livers.

HOUSSAY and SORDELLI (1921) found that rabbits, dogs and horses, whose thyroid was extirpated, furnished a greater quantity of hemolysins, agglutinins and antitoxins than controls.

ECKER and GOLDBLATT (1921) showed the necessity of an exact knowledge of anatomy in the experiments of extirpation of the thyroid and para-thyroids and ascertained that thyroidectomy with partial para-thyroidectomy did not inhibit the production of antibodies (hemolysins), whereas in the few animals who survive a complete thyro parathyroidectomy the production of hemolysins is reduced to a fiftieth of the normal.

SESTINI (1921) asserts that during immunisation against *B. typhi* the thyroid of guinea-pigs undergoes a process of hyperplasia and shows intense morphologic indications of cellular hypersecretion.

CUTLER's experiments (1922) show that the hypophysis does not have an important influence, direct or indirect, on the production and persistence of agglutinins and hemolysins in the blood, unless the part of the hypophysis which is indispensable to the survival of the animal, should not have the same influence as the whole gland.

6°) The antibodies pre-exist in the blood and liquid tissues of the body.

New ideas were advocated recently by SAHLI (1920).

SAHLI disagrees with EHRLICH in thinking the protoplasm not to be the seat of production of antibodies. The origin of these antibodies is the blood itself (which according to SAHLI is a secretion) and the liquid tissues; the cells produce the antibodies physiologically responding to stimuli from the blood and liquid tissues.

The different antibodies preexist in the blood; by the admission of the antigen (immunisation) an artificial increase is obtained and this according to the well-known law that a secretion increased to cover a deficiency exceeds the necessary quantity.

The production of antibodies would be merely a particular case of blood-regeneration, an excessive regeneration. This would take place on account of the following: Antigen and antibody unite in a colloidal combination and the function which the antibody exercised up to then ceases on that account.

The organism reacts to this loss of antibody, which had its own function, by an increased secretion, so as to produce a new and greater amount of antibodies.

II—Material and Methods of Research.

We examined the bone-marrow of 54 rabbits, some under normal conditions, most (40) of them in various stages of immunisation for the obtention of agglutinins.

The animals, killed when opportuni-

ty arose, were immediately necropsied; those which died in the course of the experimentation, were only made use of when the post-mortem could be carried out immediately after death.

In all cases the bone-marrow of the femur of both sides was examined.

The femur, once freed of the soft parts that covered it, was cut through, as near as possible to the epiphyses, with a costotome. At one end of the bone-canal I would make two incisions with sharp scissors and these would be extended carefully in such a way as to fracture the whole length of the bony tube.

Almost always one of the halves would then contain a perfect cylinder of bone-marrow. In the cases in which the consistency of the bone-marrow was reduced the results obtained were less satisfactory.

The piece of bone to which the marrow remained stuck was then placed in the fixing-fluid, or else the cylinder of bone-marrow was gently detached from the bone with the aid of pincers, and small pieces cut with the scissors were dropped successively in sublimate-alcohol and ZENKER-formol (without acetic acid), the fixing-fluid employed.

Embedding was carried out in paraffin and the sections were coloured by GIEMSA's fluid method (fixing in sublimate-alcohol) and with hæmatoxylin-eosin (fixing in ZENKER-formol).

In some cases the material was fixed only in ZENKER-formol in which cases the sections stained by GIEMSA's method were less satisfactory than the ones stained with hæmatoxylin-eosin. In other cases the only fixing-fluid employed was sublimate-alcohol; besides first-rate preparations by GIEMSA's method I obtained good hæmatoxylin-eosin ones: the staining in undiluted HANSEN's hæmatoxylin must then not exceed one minute.

I observed that shrinking was more

pronounced in material fixed in sublimate-alcohol than in material fixed in ZENKER-formol.

It is therefore more advantageous to use both fixing-fluids since the comparative study of the results obtained is then more instructive.

For certain purposes (research of fibrin, identification of reticulum-cells) I made use of various general methods (methods of MALLORY with anilin-blue and hæmatoxylin-phosphotungstic acid, V. GIESON, etc.).

Most of the animals were immunised by intravenous injections; a small number by intraperitoneal or subcutaneous ones.

In one group, the animals were inoculated on the same day with the same emulsion from the same 24 hours' culture of *B. paratyphi* A.

The emulsion was made in the following way:

Emulsion A. — I prepared 10 test-tubes, each one with 2 cc. of saline solution; in each tube I made an emulsion with a loop tested for 0,002 grs. taken from a 24 hours culture of *B. paratyphi* A; the contents of the 10 tubes were mixed in a flask which was then placed in a water-bath at 60°C. for an hours time; the contents were then shaken and 2 cc were placed in each test-tube; 10 rabbits were injected in the marginal

vein of the ear, each rabbit with the contents of one of the tubes.

In another and larger group, I inoculated each rabbit (of the weight of 950-1.500 grs) intravenously, subcutaneously and intraperitoneally with an emulsion in saline solution of 24 hours' culture in agar of *B. paratyphi* A (1 loop of 0,002 grs. 2 cc. saline solution, killed by heating during an hour in a water-bath at 65°C. The counting of white corpuscles was done in an American standard Hæmacytometer with LEVY counting chamber.

As a general rule I made 4 simultaneous determinations of the number of leucocytes, sometimes only 3 or 2, making use of the average of the figures obtained.

The normal number of white corpuscles in the rabbit is estimated at 5-14.000 (GRUBER), about 9.000 (HEINEKE), 9-12.000 (PROSCHER), 8-13.000 (TALQVIST). Recently PENTIMALLI, examining 10 normal rabbits, reported individual variations in the number of white corpuscles going from 4.520 to 10.300 per mm³ so that he advises great caution in the interpretation of slight oscillations: the figure 6.876 is the average he obtained.

My researches controlled by the histological examination of the bone-marrow, gave the following results:

Table I

Number of the animal	Weight	Nr. of white corp. per mm ³ .
Rabbit 268.....	1.500 grs.....	16.000
376.....	970 grs.....	10.697 (4 days' average)
300.....	1.060 grs.....	10.600
269.....	1.500 grs.....	7.400
		11.174 (average).

After working for some time, I was able to give SELLING's recommendation its due value, for advising one to

to observe the number of white corpuscles of each rabbit on three consecutive days and after that only making use

of the ones which did not show considerable daily oscillations (1) or an abnormal number.

The following observations of mine

(1) These oscillations rarely exceed 3—4000 white corpuscles per mm^3 of blood. The following rabbit may be taken as a normal rabbit with typical daily oscillations:

Feb. 16 th 1921—White corpuscles=9.733 per mm^3 (average of 3 determinations).

Feb. 17 th 1921—White corpuscles=11.533 per mm^3 (average).

Feb. 18 th 1921—White corpuscles=12.266 per mm^3 (average).

Feb. 19 th 1921—White corpuscles=9.256 per mm^3 (average).

demonstrate how sensitive bone-marrow is to pathological conditions, showing considerable morphologic modifications which would introduce an important cause of error into the interpretation of experiments carried out under such conditions.

I adopted the following method.

The rabbits were brought directly from the rabbit-hutches of the Institute to the laboratory and were killed as soon as the number of white corpuscles had been taken note of.

In table II are indicated the results obtained.

Table II.

Animal	Weight	Number of white corpuscles in 1 mm^3 of blood.	Killed on	Notes	Microscopic study of bone-marrow.
Rabbit 127	—	5.400	Congestion and slight oedema of reticulum.
Rabbit 128	—	34.400	Bone-marrow with normal aspect.
Rabbit 130	—	12.200	Slight oedema of reticulum; reduction in number of the parenchyma-cells.
Rabbit 271	800 grs.	(On June 3rd 1920 at 2.55 p. m.) 16.000	June 3rd 1920 at 3.10 p. m.	Killed by violent anaesthesia (Chloroform)	Bone-marrow showing pronounced oedema of reticulum, atrophy of fat-cells and foci of multiplication of myeloblasts.
Rabbit 268	1.500 grs.	(On July 17th 1920 at 2.45 p. m.) 16.000	July 17th 1920 at 3.30 p. m.	Do.	Bone-marrow with normal appearance.
Rabbit 269	1.500 grs.	(On July 17th 1920 at 3.20 p. m.) 7.400	July 17th 1920 at 3.35 p. m.	Do.	Bone-marrow with normal appearance.
Rabbit 275	—	—	Aug. 7th 1920 when agonising.	Do. Remained a few days in the laboratory getting rapidly thinner.	Pronounced oedema of reticulum, congestion, atrophy of fat-cells, regressive changes in parenchyma, abundance of pigment-cells (haemosiderin) are to be seen.
Rabbit 300	1.060 grs.	(On Sep. 2rd 1920 at 2 p. m. 2.00 p. m. 10.600.	Sep. 3rd 1920	Killed by violent anaesthesia (Chloroform).	Bone-marrow with normal aspect.

Under a strong power one notices that the most numerous cells are amphophil myelocytes and polymorphonuclear leucocytes, there being also found amongst them, looking like a diffused infiltration, small cells with nuclei very rich in chromatin and resembling blood lymphocytes in their morphology.

The megalocaryocytes are not found in reduced number; their nucleus appears well-stained and with its normal structure.

Many of the megalocaryocytes found enclose one or more leucocytes in their protoplasm.

The fat cells are found reduced in volume; the fat-containing vacuole is smaller than in similar cells of normal bone-marrow; in many cells this reduction amounts to one half or one third of normal volume, and, not infrequently, in others to one tenth of normal volume.

In cells whose vacuole is reduced to one half or one third, the nucleus retains its peripheric position and its normal appearance. The nucleus of cells reduced to one tenth of their volume is more evident, as it becomes tumid and vesicular; this kind of cell does not show pyknosis or any other aspect indicating regressive changes.

The appearance described for the fat-cells of rabbits 212 and 210 (enlarged nucleus, with a tendency to leave its excentric position and surrounded by a zone of protoplasm with an evidently reticular structure) was only rarely seen in this rabbit.

The lesions found in the bone-marrow of rabbit 214, killed 46 hrs 30 mins. after the commencement of immunisation can be seen in the following résumé:

1°) *Congestion and œdema of reticulum.*

2°) *Slight reduction in number of the cells of the medullary parenchyma; amphophil myelocytes and polymorphonu-*

clear leucocytes predominate; the last are found in their normal number, but appear more numerous on account of the reduction in number of the cells.

3°) *Dispersed infiltration of parenchyma with cells of the appearance of blood lymphocytes.*

4°) *Megalocaryocytes normal in number and in appearance many of them containing one or more polymorphonuclear leucocytes.*

5°) *Fat-cells fewer and smaller than in normal bone-marrow, with a nucleus of normal appearance; some other fat-cells very much smaller (one tenth and even less of normal volume).*

The appearance of the fat-cells I believe to indicate a stage of reconstruction or recomposition. After the loss of fat observed in the first 24 hours of immunisation and which is indicated by a special appearance of the cell (see description rabbit 212), these cells begin to regain fat-contents.

3rd. Day of Immunisation.

Rabbit 450—Weight 1.150 grs.

Killed 60 hours after inoculation.

Feb. 5 th 1920—Leucocytes: 12.15 p. m.—8000 per mm³.

Inoculated at 4 p. m. in the marginal vein of ear with 1 cc. of saline solution with a loopful (2 milligrs.) of a 24 hours' agar-agar culture of *B. paratyphi A.* in suspension. The suspension was sterilised by heating in a water-bath at 60°C. during 1 hour 30 mins.

Feb. 26 th 1920—Leucocytes (11 a. m.)=33.400 per mm³.

Feb. 27 th 1920—Leucocytes (3.30 p. m.)=34.800 per mm³.

Feb. 28 th 1920.

Found dead in the morning.

Examined under good conditions of preservation.

Autopsy—Bone-marrow soft, diffuent, of a dark red colour; in the centre (in the part corresponding to the length diameter) a dark-red and strong cord stands out from the medullary parenchyma (central vessels of bone-marrow).

Microscopical study.—Section of the material fixed in ZENKER-formol and

stained in hæmatoxylin-eosin and fluid GIEMSA.

Cellular contents of marrow approximately normal.

Capillary congestions remains very marked (Fig. 9, Pl. 21).

The œdema has suffered resorption and is only to be seen here and there in an inconspicuous manner.

The most abundant cells are amphophil myelocytes and polymorphonuclear leucocytes (Fig. 10, Plate 21); some myelocytes show mitotic figures and dispose themselves in foci.

Conspicuous foci of division of erythroblasts (normoblasts) are to be seen, some of them not only in the middle of the parenchyma, but also round the medium-sized vessels and prae-capillaries (Fig. 11, Plate 21).

The diffuse infiltration of the parenchyma by cells with the appearance of lymphocytes is evident. (Fig. 9, Plate 21).

The fat-cells show the same appearance as in rabbit 214.

Almost all megalocaryocytes contain in their protoplasm some polymorphonuclear leucocytes; some megalocaryocytes show regressive changes.

The similarities and differences between the ones here described (3rd day of immunisation) and the previously indicated ones are the following:

There is a beginning resorption of the œdema of the reticulum.

The bone-marrow is almost as rich in cells as under normal conditions, these cells are principally myelocytes and polymorphonuclear leucocytes.

Foci of regeneration of hæmoglobin-containing elements (perivascular foci of erythroblasts (normoblasts)) begin to appear.

Regeneration and replacement of fat-contents of fat-cells continues.

Lymphocytes become scarcer and lose the appearance of a diffuse infiltration.

Megacaryocytes show a pronounced phagocytic activity and undergo processes of desintegration.

3rd. Day of Immunisation.

Rabbit 216.—Weight 1,300 grs.

Inoculated in marginal vein of ear with a sterilised *B. paratyphi A.* emulsion (emulsion A) at 3.30 p. m. on April 7 th 1920.

Killed at 12.50 p. m. on April 10 th 1920 (72 grs, and 20 mins. after inoculation).

Autopsy carried out at once.

Autopsy—Bone-marrow (femur) with normal soft consistency and dark red colour. Disseminated spots of a whitish colour are to be seen.

Microscopic study.—Sections fixed in ZENKER-formol stained in hæmatoxylin-eosin and fluid GIEMSA.

Under a weak power congestion of capillaries, œdema of reticulum, reduction in parenchyma cells, disappearance of fat-cells and increase of fixed connective-tissue cells (Fig. 3, Plate 20), the latter decreased are to be observed.

A strong power shows that the dominant cells are myelocytes and leucocytes; there is also a diffused but not intense infiltration of cells with the appearance of lymphocytes.

Myelocytes are frequently found in little groups of 2, 3, 4 or 6, rarely of more cells; the most numerous groups are the ones of 4 (Fig. 6, Plate 20).

The bone-marrow shows an evident decrease in number of cells.

These cells are found close to the capillaries.

This *perivascular distribution* of marrow cells (in this case polymorphonuclear leucocytes and myelocytes) in this rabbit can be looked upon as a striking example.

The fat-cells are not in evidence; in their protoplasm no fat-containing vacuole is visible; limits of protoplasm are indistinct and merge into the œdema-fluid of reticulum. These cells are only recognisable by the structure of their

nuclei and by comparison with their appearance in other rabbits, in which transition types between these and typical fat-cells are to be found. I must call attention to the resemblance of the nucleus of these atrophied cells to those of fixed connective tissue cells.

The nucleus of fat-cells retains a typical structure in spite of the most marked alterations of the protoplasm: thus, after the total loss of the fat-contents of the protoplasm, the nucleus is only slightly more tumid and rounded and has left its peripheric position to occupy the centre of the cell. The arrangement and disposition of the nuclear chromatin is very similar to that of fat-cells from normal bone-marrow. One would be inclined to say that the important modifications of the fat-contents were physiological and not pathological.

In this bone-marrow increase in number of the fixed connective tissue cells is evident; in some places (Fig. 8, Plate 20), the parenchym is made up of 10 to 15 cells placed side by side and one behind the other. The nuclear structure of these is that of the fibroblasts; the connective tissue fibrils can be clearly distinguished.

These points where many fibroblasts place themselves side by side are not very common, however: almost always fibroblasts of characteristic appearance are seen mixed with other bone-marrow cells. All those who have studied normal bone-marrow will be well aware of the difficulty of recognising the fixed connective tissue-cells under normal conditions.

Besides small cell foci which are the foci of division of myelocytes and of hæmoglobin-containing cells (megaloblasts and normoblasts) other more extensive foci, made up of numerous densely grouped cells and contrasting markedly with the neighbouring parenchyma, are to be seen (Fig. 7, Plate 20).

Under a strong power the cells which make up the dense groups are seen to be numerous polymorphonuclear leucocytes: Many of these in one point, covering one another show a pyknotic nucleus and others are evidently undergoing complete desintegration. This point gives the impression of a small *infarct* (Fig. 7, Plate 20).

Among the densely grouped cells indicated may be seen the characteristic cells of bone-marrow parenchyma.

Condensing the facts, the bone-marrow of this rabbit, killed 72 hrs. 20 mins. after the commencement of immunisation, shows the following points of interest:

1°) *Regeneration of polymorphonuclear leucocytes (by intense division of myelocytes) is the dominant feature.*

2°) *Fat-contents of the fat cells are noticed to have disappeared completely.*

3°) *Infiltration of lymphocytes, so marked in rabbits 212 and 214, is in this case very slight.*

4°) *In this case hyperplasia of the fixed connective tissue cells is seen for the first time in this series.*

5°) *Other foci besides foci of regeneration of hæmoglobin-containing cells (perivascular foci of megaloblasts and normoblasts) are seen.*

6°) *The whole process is evidently belated, as compared to the process observed in rabbit 150, killed 60 hours after the commencement of immunisation.*

6th. Day of Immunisation.

Rabbit 407—Weight 1.030 grs.

April 28 th 1921—1.35 p. m.—Leucocytes=16 750 (average).

April 29 th 1921—1.30 p. m.—Leucocytes=10 250 per mm³ (average of 4 measurements).

May 1 th 1921—2.00 p. m.—Leucocytes=15 800 per mm³ (average of 4 measurements).

May 5 th 1921—1.40 p. m.—Leucocytes=14 230 per mm³ (average of 4 measurements).

May 6 th 1921—12.55 p. m.—Leucocytes=14 350 per mm³ (average of 4 measurements).

May 6 th 1921—Subcutaneous injection of 1 loopful of 2 milligramms of a 24 hours gelose culture of *B. pa-*

paratyphi A. in suspension in saline solution and killed in a water-bath at 62°C. during an hour.

May 12 th 1921—Rabbit was killed by violent narcosis with chloroform (death took place in 1.20 mins).

Histologic study—Under a weak power the bone-marrow is seen to be rich in cells, less so however than under normal conditions; the regularly distributed vacuoles which in normal bone-marrow correspond to fat-cells appear to be wanting.

Under a strong power, the fat cells are seen to be reduced in volume and have to a certain extent disappeared; here and there a fat-cell strongly reduced in volume and with its nucleus migrated towards the centre is to be seen; almost always the part of the reticulum round it is strongly stained by eosin.

There is no congestion.

The œdema of the reticulum is less marked than in the preceding rabbits; it is confined to certain parts of the reticulum which are intensely stained by eosin.

Of parenchyma cells, the most numerous are polymorphonuclear leucocytes which quite mask the remaining parenchyma cells.

Erythrogenetic groups are in evidence.

Megalocaryocytes with normal appearance and distribution.

Examining sections from different blocks, I noticed slight modifications of the aspect indicated; in some points there was more accentuated œdema and parenchyma cells were less numerous.

7th. Day of Immunisation.

Rabbit 218—Weight 1300 gra.

Inoculated in marginal vein of ear with 1 cc of a suspension of *B. paratyphi A.* (liquid A) at 3.30 p. m. on April 7 th 1920.

Found dead on April 4 th 1920. Necropsied when still well preserved.

Autopsy.—The bone-marrow (femur, both sides) shows firm consistence and a shining surface; colour is brick red and fine granulations, white and refringent, are to visible.

Histologic study.—Under a weak power the appearance of the bone-marrow is entirely different from the normal.

Capillaries are dilated and filled with red blood corpuscles (Fig. 13, Plate 22).

The parenchyma cells, which are numerous, show a tendency to group themselves along the capillaries (Fig. 13, Plate 22.)

Under a strong power the myelocytes are seen to be the dominant element. Not only do amphophil myelocytes constitute the majority of the cells found round the vessels but they also form groups of 4 or 6 cells in the parenchyma, between the capillaries, where they place themselves over the fat-cells (Fig. 16b, Plate 17); in the latter case they have the appearance of a group of cells (myelocytes) surrounded by a clear halo (body of the fat-cell).

Other fat-cells have approximately normal dimensions (Fig. 16a, Plate 17).

Polymorphonuclear leucocytes are scarce. Lymphocytes are not plentiful; they are found mostly round the small arteries.

The hæmoglobin-containing elements affect a grouped disposition; these groups are formed by few cells and are much less conspicuous than the ones of myelocytes; it is easy to find erythroblasts with *karyorrhexis*.

Myelocytes are as numerous as in the normal organ; many of them contain phagocytosed granulocytes.

Foci made up of big cells with an elongated nucleus and sometimes narrowing towards the middle (shape of a figure 8), with little chromatin, which I take to be connective tissues cells, are often seen in the sections. Only once and again do these foci contain any other cells besides connective tissue cells; sometimes a granulocyte sometimes non-granular mononuclear leucocytes.

Rabbit 373—Weight 1400 gra.

Feb. 4 th 1921—Leucocytes (1.25 p. m.)=16 550 per mm³ (average).

Inoculated at 1.10 p. m. on same day with 1 cc of saline solution with a loopful (itrated for 2 milligramms) of a killed 24 hours gelose culture of *B. paratyphi A.* The injection was carried out uneventfully.

Feb. 11 th 1921—At 2.00 p. m. the rabbit died. It was at once autopsied.

Autopsy.—Subject in strongly emaciated. Viscera—lungs, liver, spleen, heart and kidneys show a normal aspect. There is no pneumonia, coccidiosis lesions or myxoma.

Bone-marrow of a more or less firm consistence; it is pale in ill-defined tracts, brownish-red in others and is rather opaque than otherwise.

Histologic study.—The lesions of the bone-marrow are of the same kind as those of rabbit 218, with slight individual differences. The parenchyma cells are less abundant than in rabbit 218; there is active hyperplasia of the myeloid cells, which group themselves round the vessels and make up foci in the substance of the parenchyma.

The polymorphonuclear leucocytes, in opposition to the previous case, are very numerous, as numerous as the myelocytes.

The fat cells are noticeably smaller than normal fat-cells. Erythrogenetic groups are not conspicuous.

The lesions of the bone-marrow on the 7th day of immunisation are chiefly hyperplasia or intense proliferation of myelocytes, which group themselves round the vessels in a quite evident way; the evolution of myelocytes towards polymorphonuclear leucocytes goes on in an active way; there is individual variation with regard to the numerical prevalence of these two kinds of cells.

There is persistence of the capillary congestion; the œdema of the reticulum is inconspicuous.

The fat-cells are reduced in volume; some of them, however, have already re-

gained their size; others are hidden by the hyperplastic myelocytes.

Cells belonging to the class of the hæmoglobin-containing cells are found, but are very much less conspicuous than those belonging to the myeloid class of the myelocytes.

Small foci of fibrosis (proliferation of connective tissue cells) are plentiful, and indicate, probably, the organisation of small hæmorrhagic foci.

10th Day of Immunisation.**Rabbit 405—(1)—Weight 950 gra.**

April 11 th 1921—Leucocytes= 14 400 per mm³ (average).

April 11 th 1921—(T. 20 p. m.) Leucocytes=16 500 per mm³ (average).

April 14 th 1921—(41.10 p. m.) Leucocytes=18 450 per mm³ (average).

April 15 th 1921—(12.40 p. m.) Leucocytes=19 550 per mm³ (average).

April 16 th 1921—(12.35 p. m.) Leucocytes=15 333 per mm³ (average).

On April 16 th 1921 he was inoculated intraperitoneally with 1 loopful (2 milligramms) of a 24 hours culture on slanting gelose of *B. paratyphi A.* killed by heahring in a water-bath during an hour at 65°C.

April 26 th 1921—Killed by violent narcosis with chloroform at 2.15 p. m.

Death occured after 1.15 mins.

Autopsy—The bone-marrow did not show any macroscopic lesions.

Rabbit 408—Weight 1.140 gra.

April 28 th 1921—(1.40 p. m.)—Leucocytes=10 950 per mm³ (12 800=11 600=10 600=8 600).

May 4 th 1921—(1.30 p. m.)—Leucocytes=15 950 per mm³ (16 800=14 800=14 600=13 600).

May 5 th 1921—(2.05 p. m.)—Leucocytes=19 400 per mm³.

May 6 th 1921—(1.00 p. m.)—Leucocytes=12 850 per mm³ (13 000=12 400=11 600=10 400).

On May 6 th 1921, at 1.45 p. m., this rabbit was inoculated subcutaneously with a loopful (2 milligramms) of a 24 hours culture on gelose of a *B. paratyphi A.* in suspension in saline solution and sterilised by heating in a water-bath at 65°C. (1 hour).

May 16 th 1921—The rabbit was killed by violent narcosis with chloroform (15 mins.).

(1) This rabbit (405), which showed an abnormal number of leucocytes in the days preceding immunisation, was used nevertheless for our studies, as the lesions of the bone-marrow were perfectly similar to those found in the following rabbits which had a normal number of leucocytes.

Table III.

Rabbit	Weight	Date	Time	Number of leucocytes per mm ³ of blood.	Death	Microscopic study of bone-marrow.
375	1.180 grs.	Feb. 16th921 Feb. 17th921 Feb. 18th921 Feb. 19th921	1.00 p. m. 4.10 p. m. 4.15 p. m. 11 30 a. m.	16.400 (average of 2 determ.) 14.933 (< < 3 <) 7.133 (< < 3 <) 20.600 (< < 2 <)	Violent narco- sis (chloroform) at 1.15 p. m. on Feb. 19 th 1921.	Discrete oedema of reticulum and slight congestion. Otherwise the bone- marrow shows normal appearance.
376	970 grs.	Feb. 16th921 Feb. 17th921 Feb. 18th921 Feb. 19th921	1.15 p. m. 3.35 p. m. 4.00 p. m. 11.25 a. m.	9.733 (average of 3 determ.) 11.533 (< < 3 <) 12.266 (< < 3 <) 9.256 (< < 4 <)	Violent narco- sis (chloroform) at 1.55 p. m. on Feb. 19 th 1921.	Absolutely normal structure.
377	950 grs.	Feb. 16th921 Feb. 17th921 Feb. 18th921 Feb. 19th921	1.25 p. m. 4.00 p. m. 3.55 p. m. 11.10 a. m.	21.700 (average of 2 determ.) 17 250 (< < 4 <) 11.750 (< < 4 <) 20.666 (< < 4 <)	Violent narco- sis (chloroform); death within 2 mins, at 2.10 p. m. on Feb. 19 th 1921.	Pronounced oedema of reticulum, atrophy of fat-cells, reduction in num- ber of the parenchyma-cells (aplasia), discrete congestion.
381	1.260 grs.	March 8th921 March 9th921 March 14th921 March 15th921 March 16th921	1.10 p. m. 12.50 p. m. 3.10 p. m. 1.55 p. m. 1.55 p. m.	10.150 (average of 4 determ.) 9.800 (< < 4 <) 14.500 (< < 4 <) 13.000 (< < 4 <) 17.800 (< < 4 <)	Violent narco- sis (chloroform); death in 1.45 mins. at 4.10 p. m. on March. 16 th 1921.	Hyperplasia of parenchyma-cells (amphophil and eosinophil myelocytes with figures of karyokinesis; poly- morphonuclear leucocytes).
382	1.110 grs.	March 8th921 March 9th921 March 14th921 March 15th921 March 16th921	1.20 p. m. 1.00 p. m. 3.25 p. m. 2.05 p. m. 2.05 p. m.	10.900 (average of 4 determ.) 26.100 (< < 4 <) 13.600 (< < 4 <) 19.050 (< < 4 <) 15.700 (< < 4 <)	Violent narco- sis (chloroform); death in 1.30 mins. at 4.25 p. m. on March. 16 th 1921.	Hyperplasia of parenchyma-cells (amphophil and eosinophil myelocytes and amphophil and eosinophil poly- morphonuclear leucocytes).
383	1.200 grs.	March 8th921 March 9th921 March 14th921 March 15th921 March 16th921	1.30 p. m. 1.10 p. m. 3.40 p. m. 2.20 p. m. 2.25 p. m.	13.200 (average of 4 determ.) 8.800 (< < 4 <) 10.500 (< < 4 <) 12.350 (< < 4 <) 12.900 (< < 4 <)	Violent narco- sis (chloroform) death in 1.32 mins. at 4.33 p. m. ou March. 16 th 1921.	Normal appearance.

As the animals were submitted to close observation only some time after the work was begun, I was obliged to separate the animals into groups of very different demonstrative value.

Thus we have a Group I, in which the rabbits had a normal number of leucocytes during the term of observation and were killed without having suffered inoculation; Group II, the most important, comprehending animals under the same conditions as in Group I, but having been killed or having died in different stages of immunisation.

A Group III consists of animals that showed an abnormal number of white blood corpuscles during the term of observation.

In a Group IV, finally, I included all the rabbits that were not observed 3 days before being used for experimentation.

In this way each group serves as a control for the others, and constitutes a valuable material for comparative study.

This is a highly important point in the case of animals very sensitive to bad conditions of housing and feeding and liable to suffer from various diseases, the more so in the case of a study on a tissue like bone-marrow that shows important alterations in animals which at first sight appear perfectly normal.

Rabbits immunised Against Bac. Paratyphi A.

Rabbit	N. of animal	Injection.	Death	Term of immunisation.	Observations
1	131 A	intracardiac	Killed (by violent chloroform-narcosis.)	1 hour	Rejected: abnormal number of leucocytes on the days preceding inoculation.
2	364	intravenous	« « «	1 hour	In the bone-marrow, the most evident phenomenon is oedema of reticulum and atrophy of the fat cells, with a slight reduction in number of these cells. Congestion slight; there are well preserved polymorphonuclear leucocytes and megalocaryocytes. This rabbit is believed to have been abnormal; the only determination of the number of leucocytes was made a few minutes before injection.
3	365	«	« « «	1 hour	General appearance same as in 364. What is known about this rabbit before it was inoculated (leucocytes=64 600) leads me to think it was abnormal. Aplasia more marked than in no. 364.
4	223	«	« « «	2.20 hrs	Abnormal number of leucocytes.
5	391	«	« « «	2.38 hrs	Abnormal number of leucocytes on the days preceding inoculation; the histological study of the bone-marrow confirms the opinion that this is not a normal animal.
6	392	«	« « «	2.59 hrs	Abnormal number of leucocytes on the days preceding inoculation; the histological study of the bone-marrow confirms the opinion that this is not a normal rabbit.
7	368	«	« « «	3.34 hrs	Made use of for histological description.
8	369	«	« « «	3.57 hrs	Made use of for histological description.
9	393	«	Died	15 hs.	Spleen with intense inflammation. Rejected because it was not immediately autopsied and was undergoing cadaveric alterations.
10	397	«	«	15 hrs	Rejected: cadaveric alterations.
11	394	«	«	17 hrs	
12	210	«	«	17 hrs	Made use of for histologic description.
13	211	«	«	17 hrs	
14	372	«	«	19 hrs	The histological study of the bone-marrow confirms the observation that this is not a normal rabbit.
15	398	«	«	23.30 hrs	Rejected: autopsy made hours after death.
16	151	«	«	24 hrs	
17	152	«	«	24 hrs	
18	212	«	«	24 hrs	Made use of for histological description.

Rabbits immunised Against Bac. Paratyphi A.

Rabbit	N. of animal	Injection.	Death	Term of immunisation.	Observations
19	118 C	intravenous	Died	24 hrs	
20	131 B	«	«	24 hrs	Abnormal number of leucocytes on the days preceding inoculation; 24 hrs after inoculation pronounced leucocytosis (leucocytes=231 200 per mm ³).
21	410	subcutaneous	«	24 hrs	
22	213	intravenous	«	36 hrs	Made use of for histological description.
23	153	intraperitoneal	Killed (violent chloroform narcosis).	2 days	Pronounced (leucocytosis leucocytes=96 600 per mm ³) 24 hours after inoculation.
24	214	intravenous	«	2 days	Made use of for histological description.
25	403	intraperitoneal	Died	2 days	
26	150	intravenous	«	3 days	
27	215	«	«	3 days	
28	216	«	Killed (violent chloroform narcosis).	3 days	Made use of for histological description.
29	217	«	Died	5 days	Cadaveric alterations of parenchyma-cells.
30	367	intraperitoneal	Killed (violent chloroform narcosis).	5 days	Rejected. Abnormal number of leucocytes.
31	407	subcutaneous	«	6 days	Made use of for histological description.
32	218	intravenous	Died	7 days	«
33	373	«	«	7 days	«
34	221	«	«	8 days	Made use of for histological description.
35	222	«	«	8 days	«
36	154	«	Killed (violent chloroform narcosis).	9 days	
37	405	intraperitoneal	«	10 days	Abnormal number of leucocytes; the lesions, however, are concordant with those of normal rabbits at the same period of immunisation.
38	408	subcutaneous	«	10 days	Made use of for histological description.
39	409	«	«	10 days	«
40	133 A	intraperitoneal	«	14 days	«

III—Histopathologic Study.

4 hours of immunisation.

Rabbit 368—Weight 1.050 grs.

Inoculated on Jan. 26 th 1921 at 12.55 p. m. in marginal vein, of ear with 1 cc. of saline solution with 1 loopful (2 milligrs.) of a 24 hours' gelose culture of *B. paratyphi A.* in suspension and sterilised by heating in a water-bath at 65°C. for an hour.

Leucocytes (blood taken at 1.05 p. m.)=8 800 per mm³ (average).

Leucocytes (blood taken at 1.42 p. m.)=15 800 per mm³ (average).

Killed (violent narcosis with chloroform) at 4.44 p. m.

Autopsy.—Bone-marrow red, with whitish areas; shining surface. Consistence firm, slightly reduced. Central vein moderately turgid.

Histologic Study.—The arrangement of the parenchyma cells in groups occupying the spaces between the fat-cells is altered; the cells are disarranged and less numerous than under normal conditions. The most plentiful cells are the polymorphonuclear leucocytes; these sometimes arrange themselves round a fat cell like a crown (Fig. 14, Plate 17). Polymorphonuclear leucocytes are also to be seen occupying the territory of a fat-cell (Fig. 15, Plate 17), which would indicate a marked chemotaxis of the fat-cell for polymorphonuclear leucocytes.

The blood spaces are slightly dilated; there is also an inconspicuous oedema of the reticulum.

The fat-cells keep their normal volume.

Rabbit 369—Weight 1 200 grs.

Inoculated on Jan. 28 th 1921 at 1.000 p. m. in marginal vein of (2 milligramms) of a 21 hours' culture on slanting gelose of *B. paratyphi A.* sterilised by heating in a water-bath at 65°C. for an hour.

Number of leucocytes (counted at 1.13 p. m.)=5 000 per mm³ (average).

Killed by violent narcosis (chloroform) at 4.57 p. m. (3.57 hours after the commencement of immunisation).

Autopsy.—The bone-marrow has approximately the same appearance as in rabbit 368; its consistence is perhaps slightly firmer.

Microscopic Study.—The appearance is approximately the same as the one described in rabbit 368; slight congestion, inconspicuous oedema of reticulum, fat-cells with normal volume, disorder in the arrangement of the parenchyma-cells and abundance of polymorphonuclear leucocytes, grouping of these cells round the fat-cells; the latter have a well-preserved nucleus and some of them are entirely covered by polymorphonuclear leucocytes which appear to their surface (chemotaxis) (Fig. 15, Plate 17).

The elements of the haemoglobin-containing class of cells are in this case more abundant than in rabbit 368.

At the end of the first four hours of immunisation, the bone-marrow of rabbits 368 and 369 show a slight congestion, inconspicuous oedema of reticulum and a disorder in the arrangement of the parenchyma cells, which are less numerous than under normal conditions, and above all an intense transformation of myelocytes into polymorphonuclear leucocytes. The latter cells arrange themselves like a halo round some of the fat-cells; at other times a greater number of them make up a focus round a fat-cell. The aspects indicated would suggest a marked chemitaxis of the fat cells for polymorphonuclear leucocytes in this initial stage.

17 hours of immunisation.

Rabbit 210—Weight 1 300 grs.

Inoculated on April 7 th 1920 in marginal vein of ear with 1 cc. of a sterilised emulsion of *B. paratyphi A.* (Emulsion A).

Died during the night before 9 a. m. on April 8 th 1920.

Autopsy.—The bone-marrow (femur) has a light red colour, a shining surface and a normal consistence.

Histologic Study.—Under a weak power the section shows on a background stained by eosin a more or less uniform rose colour small circular light areas, which correspond to fat-cells; the parenchyma cells are considerably redu-

ced in number (aplasia). The capillaries are enormously dilated and full of red blood corpuscles but in a discontinuous way.

Under a strong power the scarceness, resembling disappearance of the polymorphonuclear leucocytes is made evident.

The myelocytes, which are considerably less numerous than in normal bone-marrow show pronounced regressive changes; the nucleus of many is pyknotic.

Some myelocytes make up isolated groups of 3 or 4 amphophil myelocytes; these are small foci of multiplication of myelocytes. These groups are rarely seen. The regressive changes of the myelocytes are prevalent.

A relative abundance of small cells with the appearance of lymphocytes may be noticed.

Some fat-cells have their normal dimensions; almost all, however, are reduced in volume; the nucleus is swollen and its finer structure more evident than usual, being readily stained by hæmatoxylin. In the protoplasm appears a zone where the structure is clearly reticular. This zone is found immediately round the nucleus, which occupies a much more central position than in fat-cells from normal bone-marrow.

The megalocaryocytes found are the seat of pronounced regressive changes (plasma more or less intensely stained by eosin, nucleus in frank caryolysis).

Round the degenerating megalocaryocytes are found frequently cells with the appearance of lymphocytes, but not plentifully; in the protoplasm of the megalocaryocytes, remains of phagocytosed cells.

In the bone-marrow of rabbit 210 which died not quite 17 hours after the commencement of immunisation, the lesions are as follows:

Lesions of the vessels: *pronounced congestion of blood spaces.*

Lesions of reticulum: *pronounced œdema.*

Lesions of parenchyma cells:

1) *pronounced reduction in number of bone-marrowcells (aplasia).*

2) *almost entire disappearance of polymorphonuclear leucocytes.*

3) *pronounced regressive changes in myelocytes and megalocaryocytes.*

4) *reproduction of amphophil myelocytes which constitute little groups of 3-4 cells; these foci are sparse and relatively rare.*

5) *reduction in number of the fat-cells, which show a nucleus with the finer structural details clearer than under normal conditions. This nucleus is displaced towards the centre of the cells and the protoplasm around it has a very evident reticular structure.*

6) *relative abundance of cells morphologically identical to lymphocytes (diffuse infiltration of lymphocytes).*

24 hours of immunisation.

Rabbit 212—Weight 1 300 grs.

Inoculated on April 7th 1920 at 3.30 p. m. in marginal vein of ear with 1 cc. of a killed emulsion of *B. paratyphi A.* (Emulsion A). Died after 9.00 a. m. on April 8th 1920. Autopsied before 1.00 p. m.

Autopsy.—The bone-marrow is of a dark red colour; small points of a darker red may be seen in it.

Its consistence is slightly diminished. The surface is shiny.

The central vein is voluminous, turgid, looking like a cord of red colour, easily separated from the parenchyma of the bone-marrow.

Histologic Study.—Under a weak power the bone-marrow shows an evident reduction in the number of the parenchyma cells (Fig. 3, Plate 19).

The reticulum is the seat of a pronounced, generalised œdema (Fig. 4, Plate 19).

The capillaries are dilated and filled with red blood corpuscles (Fig. 5, Plate 19) between which normoblasts,

a few rare myelocytes and leucocytes may also be seen.

Under a strong power the study of the parenchyma revealed the following facts:

Great scarcity, or almost complete disappearance of the polymorphonuclear leucocytes is to be seen.

Cells with the appearance of lymphocytes are particularly abundant (Fig. 4, Plate 19); these cells appear isolated and never form groups of more than 2-3 cells.

Besides the lymphocytes, which, in certain fields appear to be the dominating element, myelocytes are to be seen.

Some myelocytes show a nucleus well-stained by haematoxylin, very often apparently two-lobed.

Some myelocytes possess two circular nuclei of unequal size; agglomerations of 4 and more cells are not rare; each cell shows, then, a two-lobed nucleus or possesses two spheric nuclei always of unequal size (forms of division of the myelocytes).

Besides the above described myelocytes other not uncommon ones show different lesions. In some, the nucleus appears frankly stained, in a diffuse way, like the shadowing of a normal nucleus (caryolysis); in others, the nucleus is reduced to a black-coloured condensed mass, or to small spheric masses (3-5) of unequal size, strongly stained (*caryorrhesis* and *pycnosis*).

The volume of almost all the fat-cells is reduced as compared with a fat-cell of normal bone-marrow (Fig. 3, Plate 68).

The nucleus occupies a very much more central position than it would in a normal fat-cell (Fig. 4, Plate 68); it is never perfectly central but always slightly excentric also more voluminous than in normal fat-cells, ovoid in shape, and poor in chromatin. It occupies a zone of protoplasm with a reticular structure. In the meshes of the reti-

culum, round the nucleus, are seen little vacuoles. In the peripheric part of the protoplasm the vacuoles are bigger, separated by fine septa from the reticulum.

In the immediate neighbourhood of the fat-cell a substance of uniformly rose colour is seen in preparations stained by hæmatoxylin-eosin (fluid transudate of œdema).

It is not possible to find a megalocaryocyte with a normal appearance; these cells are reduced in number and have different appearances.

In one case, the volume is approximately that of a normal megalocaryocyte. The nucleus shows the characteristic configuration. It stains, however, very little by hæmatoxylin, looking like a shadow of dark blue colour, bigger than a normal nucleus; no fine details of structure are seen, in it, the mass having on the contrary, a homogeneous and uniform appearance.

The protoplasm is made up of minute, densely grouped granulations, of uniform, *sic*, frankly coloured rose-red by the hæmatoxylin-eosin and pale blue by GIEMSA's fluid process.

In another, aspect, the volume of the cell is enormously reduced, sometimes to one half the normal size. The megalocaryocyte has in this case the dimensions of a myelocyte (Fig. 5, Plate 19).

The nucleus appears as a condensed mass, strongly stained in very deep blue; it does not show fine structural details.

The granular protoplasm forms round the condensed nucleus a more or less narrow zone; it shows the granular structure described above.

The lesions of the bone-marrow 24 hours after the commencement of immunisation are, therefore:

1) *aplasia* (reduction of the number of cells).

2) *relative abundance of cells with the morphology of lymphocytes which*

are either found in the interior of the blood spaces or else are in the reticulum where they appear as isolated elements.

3) reduction in number, almost absence, of polymorphonuclear leucocytes.

4) regressive changes in the myelocytes, megalocaryocytes and fat-cells.

5) proliferation of myelocytes, with not infrequent disseminated foci, made up of 4 or more of these cells.

Probable interpretations.

The lesions seen in the bone-marrow of rabbits 210 and 212, which died respectively 17 and 20 hours after the beginning of immunisation, are of the same nature.

Small variations of intensity of the lesion, only, are to be seen.

Thus in rabbit 210, the œdema of the reticulum is more accentuated than in 212. In rabbit 212 the cells with the appearance of lymphocytes are still more numerous and diffusely distributed than in rabbit 210.

These lesions are probably due to the following:

The conspicuous reduction in leucocytes is to be explained, naturally by the leucocytosis observed in this phase of immunisation.

The multiplication or regeneration of the myelocytes is due to this same leucocytosis.

The congestion of the capillaries and œdema of the reticulum are directly dependant on the introduction into the circulation of the antigen (dead bacteria), certainly bringing with it toxins (endotoxins). The congestion must precede the œdema which is only the consequence of an intense and lasting congestion. We had, besides, the opportunity to see the ease with which, in various pathological conditions, the œdema of the reticulum of the bone-marrow produces itself, and which is explained by the special structure of the capillaries of the bone-marrow.

As to the regressive changes seen

in the myelocytes, megalocaryocytes and fat-cells, I think that the vascular lesions (œdema and congestion), bringing with them modifications in the cellular metabolism, are in themselves sufficient explanation; it is not possible, however, to exclude even here an action of the antigen.

The explanation of the abundance of cells with the morphology of lymphocytes offers some difficulty; the same fact had already been observed by SELLING in the regeneration of bone-marrow after benzol intoxication; it is most probable that it should be a case of emigration of these cells from capillaries of the bone-marrow where they are also to be seen in the sections (Fig. 4, Plate 19).

36 hours of immunisation.

Rabbit 213—Weight 1.300 grs.

Inoculated in marginal vein of the ear with 1 cc. of a killed emulsion of *B. paratyphi A.* (Emulsion A) at 3.30 p. m. on April 7 th 1920.

Died after 9.00 a. m. on April 8 th 1920. Autopsied at 1.00 p. m. on same day.

Autopsy—The bone-marrow has a lightred colour. The consistence is diminished, the organ is easily dilacerated, leaving pieces sticking to the bone when the rest is removed. The central vessels are not very conspicuous.

Histologic Study—The lesions of the bone-marrow of rabbit 213, died at the 36th hour of immunisation, are of the same kind as those seen in rabbit 212.

The differences are:

- 1) The congestion is less pronounced.
- 2) The proliferation of myelocytes is more pronounced, figures of indirect division are frequent.
- 3) Cells with pigment are found in greater number.

2nd Day of immunisation.

Rabbit 214—Weight 1 300 grs.

On April 7 th 1920 at 3.30 p. m., inoculated in the

marginal vein of ear with 1 cc. of a emulsion *B. paratyphi* A. (emulsion A).

Killed on April 9 the 1920 at noon (46.30 hrs after inoculation).

Autopsy was made immediately.

Autopsy—The general colour of bone-marrow (femur) was red. The surface brilliant. The consistence normal.

Histologic Study—Sections of material fixed in ZENKER-formalin, stained by hæmatoxylin-eosin and fluid GIEMSA.

Under a weak power a pronounced congestion of the small vessels (capillaries), œdema of the reticulum and reduction in volume and number of the fat-cells.

The parenchyma appears to be poorer in cells than the normal; this is not, however, marked.

Areas of necrosis are not to be seen.

Rabbit 409—Weight 1 130 grs.

May 5 th 1921—(2.00 p. m.) Leucocytes=10 550 per mm³ (11 600—11 200—10 000—9 400).

May 6 th 1921—(1.15 p. m.) Leucocytes=12 800 per mm³ (14 800—13 400—11 800—11 200).

May 6 th 1921—At 1.45 p. m. inoculated subcutaneously with 1 loopful (2 milligramms) of a culture in gelose of *B. paratyphi* A. in suspension in saline solution and sterilised by heating in a water bath at 65°C for an hour.

May 16 th 1921—Killed, at 4 00 p. m. by violent narcosis (chloroform).

One description alone will suffice for these three rabbits, so similar are the lesions found.

The only difference is that cells with pigment are found only with difficulty in rabbits 405 and 408 whereas they are not uncommon in 409.

Histologic Study—Under a weak power the fat-cells are seen to be reduced in size and the parts of the reticulum which surround them are strongly stained by eosin. In preparations by MALLORY's process (anilin blue) these parts are uniformly coloured orange red, giving the appearance of hyaline substance, whereas the remainder of the reticulum stains blue (œdema fluid).

The parenchyma cells are practically as numerous as in normal bone-marrow.

A really evident feature of this bone-marrow, which agrees with BUNTING and SELLING's descriptions of the regenerations of the bone-marrow, is the existence of myelocytes in small islets of 3, 4, 6 or 10 and more cells, side by side with other different groups of hæmoglobin-containing cells (groups in which there is regeneration of cells of the myeloid class and erythrogenetic groups).

The polymorphonuclear leucocytes are also abundant and the megalocaryocytes are well preserved.

Ocasionally discrete areas with capillary congestion are seen.

The aspect of these lesions is not uniform; in some places the œdema of the reticulum is more pronounced and the fat-cells are less voluminous; the parenchyma cells, although less numerous, show a disposition in distinct-groups of cells of the myeloid series and erythrogenetic cells.

The bone-marrow of the 10th day of immunisation shows an intense regeneration of the parenchyma cells, of the cells the myeloid series as of the hæmoglobin-containing cells; the former more numerous, however, than the latter.

The congestion of the capillaries exists only in rare places and the œdema of the reticulum is already being reabsorbed; in the immediate neighbourhood of the fat-cells is found a homogeneous substance, staining itself in orange red by MALLORY's method (anilin blue) and staining strongly with eosin (hyaline substance?).

14th Day of immunisation.

Rabbit 133 A—Weight 1.750 grs.

Jan. 19 th 1920—Leucocytes=13 290 per mm³.

Jan. 20 th 1920—Leucocytes (4 00 p. m.); 6 600 per mm³. Intraperitoneal inoculation (4 20 p. m.) of 1.5 cc. of a mixture of 2 cc. of saline solution with 1 cc. of 24 hours broth culture of *B. paratyphi* A.

Jan. 21 rst 1920—Leucocytes (2.00 p. m.)=18 800 per mm³.

Jan. 22 nd 1920—Leucocytes (12.30 p. m.)=11 800 per mm³.

Jan. 24 rd 1920—Inoculated peritoneally (1.30 p. m.) with 3 cc. of saline solution with 2 loopfuls of a 24 hours culture on gelose of *B. paratyphi A.*

Feb. 3 rd 1920—Leucocytes (11.00 a. m.)=13 000.

Killed at 1.30 p. m.

Histological Study—Under a small power the bone-marrow is seen to be richer in cells than the normal organ. The clear spaces that correspond in the latter to fat-cells are in our case unrecognisable; in their place numerous parenchyma cells diffusely disseminated are to be seen.

The blood-capillaries are not dilated and can be recognised without difficulty (under a weak power) between the numerous parenchyma-cells.

Under a strong power one observes that the most numerous cells are myelocytes and polymorphonuclear leucocytes; it is quite common to see foci of myelocytes and round the margins of these numerous polymorphonuclear leucocytes. Amongst these, it is easy to find megalocaryocytes, almost all of them with 1 or 2 phagocytosed leucocytes in their plasma; cells of the hæmoglobin-containing series are also found.

The most remarkable fact, however, of this bone-marrow in marked hyperplasia is the existence of foci of proliferation of the reticulum cells, which form structures at first sight similar to lymphoid follicles (Fig. 17, Plate 17).

The foci are fairly conspicuous, even under a weak power, on account of the absence of granulocytes in them; they are made up of big cells with a nucleus round or oval, poor in chromatin, showing 1, to 3 nucleoli, protoplasm slightly basophil (reticulum cells); staining by anilin blue (MALLORY) shows that these do not contain any connective tissue fibrils; in certain favourable fields one sees that the protoplasm of these cells has fine, strands of protoplasm

which go out towards similar ones from other cells. These cells have phagocytic activity; their protoplasm sometimes holds desintegrating granular leucocytes, round granules stained by eosin and sometimes light yellow pigment. In the foci, there occur other cells amongst these; they have a round nucleus, with abundant chromatin and a protoplasm without granulations; some have the morphology of lymphocytes.

When the focus becomes larger, the big cells occupy the central part, imitating the germ centre of a lymphoid follicle with its lymphoblasts; the non-granular mononuclear cells and lymphocytes, becoming more numerous, occupy the marginal zone; the appearance reminds one of a lymphoid follicle (see Fig. 17, Plate 17).

The knowledge we have of the reticulum cells of hæmatopoietic organs is not yet definite. Some authorities, like DOWNEY and WEIDENREICH, admit the formation of mononuclear leucocytes and lymphocytes at the cost of the reticulum of lymphoid organs.

I do not wish to assert in a categorical way that the foci I described are lymphoid organs; that would be the subject of another research. What I should like to state clearly, is the difference between these foci, which are perhaps lymphoid follicles, and the foci of proliferation of connective tissue cells (fibrosis) which were met at every moment in this series of rabbits from the 6th day on, and that indicate small hæmorrhagic centres under way of organisation.

ASKANAZY thinks that under normal conditions the bone-marrow of children contains lymphoid follicles, which SCHRIDDE denies, assuming that this can happen only under pathological conditions. The lymphoid follicles indicated by ASKANAZY showed no germ-centres.

If in this rabbit, these should be real lymphoid follicles, which I think

very probable, this fact would have a special interest, since it would show the possibility of lymphoid follicles with a quite evident germ-centre occurring in the bone-marrow (Fig. 17, Plate 17).

In normal rabbits only occasionally did I find lymphocytes, which were always isolated. The existence of lymphoid follicles in the bone-marrow of the adult rabbit is, according to me, a pathological condition. Might not the expression of «*lymphoid metaplasia*» in this case be not altogether unsuitable.

IV—Conclusions.

It is quite common to observe histopathological lesions of the bonemarrow of apparently healthy rabbits; of these, however, the number of leucocytes is almost always abnormal. SELLING's recommendation, i. e. to observe during a period of 3 consecutive days the number of leucocytes and exclude the rabbits that, during this time show considerable daily variations or an abnormal number, proved very useful.

I carried out a histopathological study on the bone-marrow of 40 rabbits immunised for the obtention of agglutinins (*B. paratyphi A.*)

I ascertained the existence of lesions succeeding each other with great regularity; this process can be divided according to its morphological characters into the following phases:

4 hours of immunisation.

Slight congestion, inconspicuous œdema of reticulum and a modification (disorder) in the normal arrangement of the parenchyma cells which are less numerous than under ordinary conditions and above all an intense transformation of myelocytes into polymorphonuclear leucocytes. These polymorphonuclear leucocytes, dispose themselves after the fashion of a crown round some fat-cells and make up a little

focus round one of them (s. Figs. 11 and 15, Plate 17). The aspects observed show a pronounced chemotaxis of the fat-cells for the polymorphonuclear leucocytes.

17 to 36 hours.

Marked congestion and pronounced œdema of reticulum (s. Fig. 3, Plate 19). Considerable reduction in the number of parenchyma cells (aplasia) (s. Figs. 3 and 4, Plate 19), with almost complete disappearance of the polymorphonuclear leucocytes. Pronounced regressive changes in the myelocytes and megalocaryocytes (Fig. 5, Plate 19). Multiplication of amphophil myelocytes, in its first stages, constituting little foci of 3-4 discrete and rare cells.

Reduction in volume of fat-cells (v. Plate 19, Fig. 3), whose nucleus showing the fine structural details, is slightly tumefied and displaced towards the centre of the cell (v. Plate 19, Fig. 4); the protoplasm all around has a clearly reticular structure. Finally, a diffuse infiltration of leucocytes (v. Plate 19, Fig., 4).

2nd Day.

There is persistence of the congestion, œdema of reticulum, and diffuse infiltration of leucocytes, also regeneration of the polymorphonuclear leucocytes and perhaps a slight excess of them. Reconstitution of the fat-contents of the fat-cells.

3rd Day.

The resorption of the œdema of the reticulum begins. The congestion of capillaries persists (Plate 21, Fig. 9). The parenchyma cells, as abundant as in the normal state, are chiefly myelocytes, disposed in small foci of 2, 4 or more cells (Plate 20, Fig, 6) and polymorphonuclear leucocytes. There begin to appear foci of regeneration of the hæmo-

globin cells (perivascular foci of erythroblasts (normoblasts) (v. Plate 21, Fig. 11).

The reconstitution of the fat-contents of the fat-cells continues (v. Plate 21, Figs. 9, 10 and 11). The lymphocytes become rarer, the character of a diffuse infiltration which they showed disappears; megalocaryocytes in pronounced phagocytic activity. Discrete hyperplasia of the fixed connective tissue cells (fibrosis) (v. Plate 20, Fig. 8).

5th, 6th and 7th Days.

The congestion of the capillaries shows a tendency to disappear and the œdema of the reticulum is becoming reabsorbed. Hyperplasia or intense proliferation of myelocytes, grouped round the vessels in a conspicuous manner (Plate 22, Fig. 13 and Plate 17, Fig. 16); active evolution of myelocytes into polymorphonuclear leucocytes, with individual variations with regard to the numerical predominance of one kind of cell over the other. Fat-cells reduced in volume; some of them, however, have already reacquired their original dimensions (Plate 22, Fig. 12); others are hidden by the myelocytes in hyperplasia (v. Plate 17, Fig. 16). Numerous small foci of fibrosis (proliferation of connective tissue cells) probably representing the organisation of small hæmorrhagic foci (v. Plate 20, Fig. 7).

7th Day.

Capillary congestion only in a few points, œdema of reticulum has already undergone resorption; round the fat-cells there has deposited itself a substance with the characters of hyaline substance. Parenchyma with an active regeneration of cells, not only of the more numerous cells of the myeloid series, but also of the hæmoglobin-containing elements.

14th Day.

Bone-marrow in marked hyperplasia, the most numerous cells being myelocytes and polymorphonuclear leucocytes. Owing to the hyperplasia of the cells of the myeloid series, the fat-cells are not conspicuous. A specially interesting fact is the existence of foci with the structure of lymphoid follicles and possessing a germ-centre (v. Plate 17, Fig. 17). Megalocaryocytes with pronounced phagocytic activity.

Condensing the facts, we may say that in the course of immunisation for the obtention of agglutinins, the bone-marrow undergoes, right in the first hours, a marked reduction in the number of its cells, with intense congestion and œdema of reticulum and regressive alterations in the parenchyma-cells. A remarkable fact is the subsequent loss of the fat-contents of fat-cells, preceded by the disposition of polymorphonuclear leucocytes round them like a crown. The reduction in volume of the fat-cells appears to be frequent in different pathological states of the bone-marrow, but the chemotaxis of polymorphonuclear leucocytes for the fat-cell had not yet been described and only subsequent research will show whether it is a lesion peculiar to bone-marrow or not.

After this initial period, there is gradual regeneration of the different cells; before this the bone-marrow is the seat of an infiltration by cells with all the characters of blood-lymphocytes; this invasion precedes the phase of regeneration of the myelocytes and cells of the hæmoglobin-containing series and this curious observations might be of great theoretic importance.

The fat-contents of fat-cells are gradually rebuilt.

Later on, on the 6th day of immunisation, the most noticeable histologic alteration is an intense hyperplasia of the parenchyma-cells; mitotic figures of di-

vision of the myelocytes are to be seen in great abundance. This hyperplasia becomes marked on the 10th and 14th days; the fat-cells are then hidden by the parenchyma-cells. At the same time there is a regeneration, on a smaller scale of the cells of the hæmoglobin containing series. At this time, a fact of general interest is the appearance of formations morphologically similar to lymphoid follicles with a germ-centre.

It is interesting to compare the curve of agglutinin-production with the lesions in the bone-marrow. This curve attains its maximum on the 6th 8th days of immunisation (TSUKAHARA), coinciding thus with a marked hyperplasia of the cells of the myeloid series and with the regeneration of the fat-content of the fat-cells of the bone-marrow.

Doubtlessly the lesions I observed are closely connected with the well-

known modifications of the blood in immunisation.

I think that the action of the antigen, leucocytosis and the production of antibodies are facts closely connected among themselves and that all of them concur towards producing the lesions of the bone-marrow seen during immunisation.

This is not a gratuitous supposition since HEKTOEN's experiments have shown the «leucocytogenic-centres» to take part in the elaboration of antibodies and TISCORNIA, employing a special technic isolated from the leucocytes of immunised animals substances with immunising properties.

Taking into account recent immunologic research and experiments with physical agents such as X-rays, radium and thorium X, all of which tend to point out the bone-marrow as seat of production of some antibodies (agglutinins), we may conclude that the morphological descriptions submitted in this work confirm this point of view.

Explanation of Figures.

Figs. 1 to 13, Plates 18 to 22, are microphotographs of microscope sections of rabbit bone-marrow stained with hæmatoxylin-eosin.

Figs. 14 and 15, Plate 17, are drawings from microscopic sections of bone-marrow stained with hæmatoxylin-eosin and drawn with ZEISS' Comp. Oc. 4 and Homog. Imm. Obj. 1/12; Fig. 16 is drawn with ZEISS' Oc. 2 and Homog. Imm. 1/12. Fig. 17, Plate 17, is drawn from a section of bone-marrow stained by GIEMSA's fluid process and examined with ZEISS' Comp. Oc. 6 ZEISS Obj. DD.

Plate 17.

Fig. 1 — Bone-marrow at the 4th hour of immunisation (rabbit 369).

Leucocytes placed round fat-cells like a halo (chemotaxis of the fat-cell for leucocytes).

Fig. 2 — Bone-marrow at the 4th hour of immunisation (rabbit 369).

Leucocytes grouped round fat-cell are more numerous than in Fig. 14, and cover the fat-cells

Fig. 3 — Bone-marrow at the end of the 7th day of immunisation (rabbit 218), showing an active proliferation of myelocytes.

Observe the predomination of this kind of cell over the other parenchyma-cells and its grouping round the blood-vessels. Capillaries dilated, slightly less so than in the first days of immunisation. Some fat-cells are approximately normally-sized (a); others are hidden by groups of myelocytes giving at first sight the impression that they had been invaded by myelocytes (b.)

Fig. 4 — Bone-marrow at the end of

the 14th day of immunisation (rabbit 133-A).

Cell-focus that must be identified with a lymphoid follicle with its germ-centre.

The central part (germ-centre) is occupied by big cells of a basophil cytoplasm, oval nucleus with little chromatin and with 1—2 nucleoli (*reticulum-cells*).

Peripherically there are numerous cells with the morphology of lymphocytes, among which are to be observed also cells of the marrow-parenchyma (myelocytes and leucocytes).

In these cell-foci erythroblasts are not seen.

Plate 18.

Fig. 1 — Normal rabbit bone-marrow (seen under a weak power).

The clear spaces correspond to fat-cells; between the latter are arranged the parenchyma-cells. Blood-vessels are not in evidence.

Fig. 2 — Normal rabbit bone-marrow (seen under a strong power).

Plate 19.

Fig. 3 — Bone-marrow after 24 hours of immunisation (rabbit 212).

Pronounced congestion and oedema of reticulum (as compared with Fig. 1, Plate 18). Reduction in number of parenchyma-cells (aplasia). Fat-cells reduced in volume.

Fig. 4 — Bone-marrow after 24 hours of immunisation (rabbit 212).

(To be contrasted with Fig. 2, Plate 18).

Reduction in number of parenchyma-cells (aplasia). Abundance of cells with the morphology of lymphocytes. Congestion and oedema of reticulum. Lesions of fat-cells (cf. text).

Fig. 5—Bone-marrow after 24 hours of immunisation (rabbit 212). Megalocaryocytes with regressive changes (cf. text).

Plate 20.

Fig. 8—Bone-marrow at the end the 3rd day of immunisation (rabbit 215).

Intense proliferation of myelocytes, forming small groups of 3 or 4 cells. Diffuse infiltration of lymphocytes. Complete disappearance of fat-contents of fat-cells.

Fig. 9—Bone-marrow at the end of the 3rd day of immunisation (rabbit 216.)

Foci of polymorphonuclear leucocytes; many of them superposed on one point show a pyknotic nucleus and other ones are in complete desintegration (a small infarct).

Fig. 10—Bone-marrow at the end of the 3rd day of immunisation (rabbit 216).

Increase in number of fixed connective tissue cells which are seen 10-15 at a time placed side by side and in rows.

Plate 21.

Fig. 9—Bone-marrow at the end of the 3rd day of immunisation (rabbit 150).

Marked congestion (compare with Fig. 1, Plate 18). Parenchyma-cells, although less numerous than in normal marrow, are more numerous than in the first 24 hours of immunisation. (compare with Fig. 3, Plate 19).

Fig. 10—Bone-marrow at the end of the 3rd day of immunisation (rabbit 150).

Foci of regeneration of hæmoglobin-containing elements [perivascular foci of erythroblasts (normoblasts)].

Fig. 11—The same section as in figure 9 seen under a higher power.

The most abundant parenchyma-cells are myelocytes.

Plate 22.

Fig. 12—Bone-marrow at the end of the 5th day of immunisation (rabbit 367).

Hyperplasia of parenchyma-cells, of which the most numerous are polymorphonuclear leucocytes. In the fat-cells can be observed regeneration of fat-contents. Oedema of reticulum largely reabsorbed.

Fig. 13—Bone-marrow at the end of the 7th day of immunisation (rabbit 218).

Pronounced congestion and grouping of parenchyma-cells round blood-vessels.