EXPERIMENTAL STUDIES UPON LYMPHOCYTES.

I. THE REACTIONS OF LYMPHOCYTES UNDER VARIOUS EXPERIMENTAL CONDITIONS.

BY ALWIN M. PAPPENHEIMER, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

Hunger, chronic inanition, infections, or such specialized forms of injury as those caused by the x-ray, all bring about a sudden or gradual destruction of the small thymus cells. We have no knowledge as to the significance of the fragility of thymus lymphocytes, nor is anything known of the more intimate factors concerned. Compared with the detailed studies bearing upon the biological behavior of the red blood cells, our knowledge of the thymus cells and of the reactions of lymphocytes in general to environmental conditions is meager.

The fact that we have had no sharp criterion of injury, comparable to the hemolytic reaction, and available for comparative quantitative studies, is probably one of the reasons for this. Morphological changes in film preparations or tissues are often difficult to interpret. The cessation of ameboid activity, which has been used by Christian and Leen[1] and others as an index of cell injury, in the study of leukotoxins, is hardly applicable in the case of the sluggish and often entirely immobile lymphoid cell.

Since the introduction into common use of trypan blue and other vital stains, it has been repeatedly observed that the injured or dead cell reacts to these dyes by a diffuse staining of the entire cell, including the nucleus. Advantage has been taken of this fact by Gross[2] in his work on experimental nephritis, by

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[1] Christian, H. A., and Leen, T. F., Some Further Observations on Leucocytotoxins, *Boston Med. and Surg. J.*, 1905, clii, 397.

[2] Gross, W., Experimentelle Untersuchungen über den Zusammenhang zwischen histologischen Veränderungen und Funktionsstörungen der Nieren, *Beitr. path. Anat. u. allg. Path.*, 1911, li, 528.

MacCurdy and Evans[3] in their studies of the cytopathology of experimental poliomyelitis, and by Rous and Jones[4] in their work on the protection of pathogenic organisms by living tissue cells. Evans and Schulemann[5] state that mechanical injury to leukocytes suspended in trypan blue causes instant diffusion of the dye into the traumatized cells.

It occurred to us that use could be made of this diffuse staining of injured cells to test the effect of various agencies upon the lymphocytes *in vitro*. A simple and satisfactory technique has been developed to this end. Our studies have been concerned chiefly with the small thymus cells of the rat, which provide readily available material, but we have applied the method also to lymphocytes from freshly removed human tonsils.

The method of procedure is as follows: The freshly excised thymus is teased with small forceps, the rat having been previously exsanguinated by aspirating blood from the heart, under ether anesthesia. There is obtained in this way a milky suspension composed entirely of small thymus lymphocytes, and practically free of red blood cells and fixed reticular elements. Suspensions of tonsil lymphocytes are prepared in a similar way, although it is more difficult to obtain them wholly blood-free.

The cell suspensions are then subjected to various experimental influences, and after a fixed period, a solution of trypan blue in Locke's solution is added in known dilution, which, however, may be purposely varied to meet different conditions. When the cells were suspended in Locke's fluid, a dilution of 1:10,000 gave the best results; in the presence of serum or other colloids, a dilution of 1:5,000 is preferable. A drop of the stained suspension is then placed in a counting chamber, and the percentage of stained cells immediately determined by counting from 300 to 600 cells with the low power.

There is generally no difficulty in detecting with No. 3 objective the sharply stained, non-refractile blue cells from the highly refrac-

[3] MacCurdy, J. T., and Evans, H. M., Experimentelle Läsionen des Centralnervensystems, untersucht mit Hilfe der vitalen Färbung, *Berl. klin. Woch.*, 1912, xlix, 1695.

[4] Rous, P., and Jones, F. S., The Protection of Pathogenic Microorganisms by Living Tissue Cells, *J. Exp. Med.*, 1916, xxiii, 601.

[5] Evans, H. M., and Schulemann, W., The Action of Vital Stains Belonging to the Benzidine Group, *Science*, 1914, xxxix, 443.

tile unstained lymphocytes. Under certain conditions, however, various degrees of transitional staining are encountered which make the determinations uncertain. This is particularly the case when the proportion of stained cells is high. There is then a tendency for all the cells to become gradually stained, so that a repetition of the count may give a much higher proportion of stained cells. On the other hand, when the proportion of stained cells is low, the time element becomes a less important factor.

Although these variations are at times disturbing and make it difficult to obtain coherent results, the method has proven of service, and by following a routine mode of procedure, the accidental fluctuations have been greatly reduced. We have repeatedly obtained duplicate counts varying less than 1 per cent, and in general the variations inherent in the method probably do not exceed 5 per cent, except when the proportion of stained cells is high.

Using this technique, we have systematically studied the reactions of the thymus cells under a fairly wide range of experimental conditions. The primary purpose was to test the availability of the method, so that our observations in many cases are merely the starting-point for a more thorough study of the questions involved.

The Effect of Hypertonic and Hypotonic Solutions.

Suspensions of thymus and tonsil lymphocytes were exposed to salt solutions of various concentrations, and the percentage of stained cells after the addition of trypan blue was determined. Locke's fluid was rendered hypotonic by the addition of distilled water, and the same test was applied.

The optimum concentration was found to be between 0.8 and 0.9, an increase in the percentage of stained cells being noted both with hyper- and hypotonic solutions. Hypotonic solutions of Locke's fluid were better borne than corresponding dilutions of pure sodium chloride.

The Effect of Varying Hydrogen Ion Concentration.

Fragments of thymus from the anesthetized rat were teased in balanced solutions of 0.093 M Na_2HPO_4 and KH_2PO_4, the hydrogen

ion concentration being varied from P_H 8.0 to P_H 6.0. The isotonicity of the solutions was tested against rat blood corpuscles. The percentage of stained cells upon adding trypan blue was determined after 1 to 2 hours. The time factor was checked, as far as possible, by counting the extremes of the series first, so that variations due to the longer exposure would affect especially the cells in solutions of approximately the hydrogen ion concentration of the blood.

Three repetitions of the experiment gave substantially the same result. The curves obtained, while not mathematically exact, probably represent fairly well the actual conditions. The optimum hydrogen ion concentration, or the concentration at which the minimum number of cells show diffuse staining, lies between P_H 6.8 and P_H 7.2. The variations between P_H 7.0 and P_H 8.0 are very slight, so we may infer that the fluctuations occurring during life in the circulating blood do not greatly affect the thymus cells. It is interesting to note that the optimum concentration, P_H 6.8 to P_H 7.2, is decidedly more acid than that of the circulating blood, but corresponds with the hydrogen ion concentration of the tissues as estimated by Michaelis.[6]

Beyond P_H 6.8, there occurred in all the experiments a rapid increase in the percentage of stained cells. At P_H 6.0 or P_H 6.2, practically all the cells stain diffusely. At the alkaline end of the series plasmolysis of the cells occurred and the counting of the cells became difficult. The rise in the percentage of stained cells is not so marked as with the acid solutions and was not obtained at all in one of the experiments.

Text-figs. 1 and 2 are typical of the results obtained.
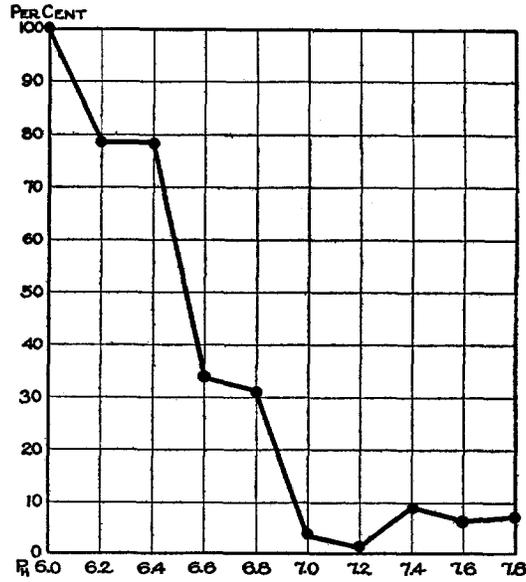
The Effect of Temperature.

The effect of temperature was tested by immersing suspensions of thymus cells successively in small test-tubes in a water bath. Each tube was kept for 10 minutes at the desired temperature, and the proportion of stained cells immediately counted (Text-fig. 3).

It is seen that between 36° and 48°C. there is little variation in the percentage of stained cells. At 48°C. there is an abrupt rise in the curve, and at 51°C. practically all the cells are stained. The
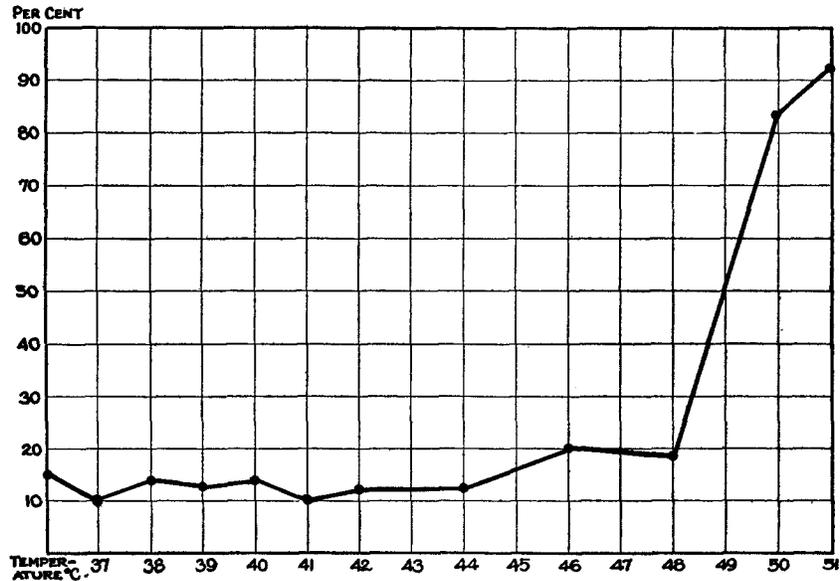

[6] Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 1914.
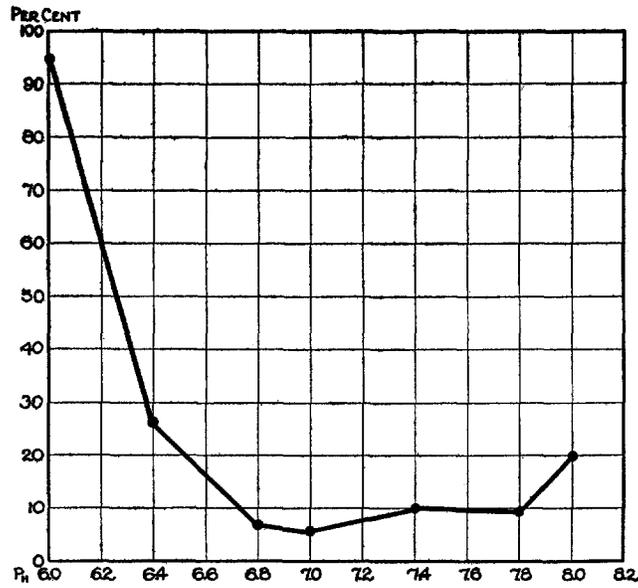
TEXT-FIG. 1. Experiment 1. The effect of phosphate solutions of varying hydrogen ion concentration upon the stainability of rat thymus cells.

TEXT-FIG. 2. Experiment 2. The effect of phosphate solutions of varying hydrogen ion concentration upon the stainability of rat thymus cells.

TEXT-FIG. 3.   Experiment 3.   The effect of temperature upon the stainability of rat thymus cells.
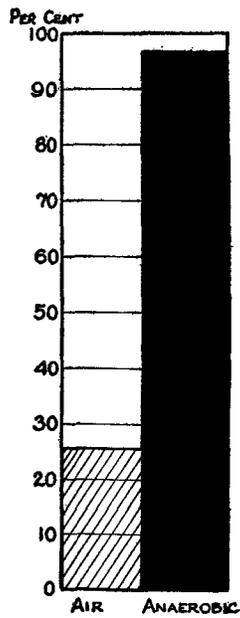
TEXT-FIG. 4.   Experiment 4.   The effect of asphyxia upon the stainability of rat thymus cells.

cells become irregular in shape and stain intensely. There is no diminution in their number. The curve suggests that the thermal death point lies between 48° and 51°C.

We have found no reference to the effect of heat upon lymphocytes, but Schultze[7] gives 50°C. as the temperature at which leukocytes (polynuclears?) undergo heat rigor.

Cells left over night in the ice box (temperature approximately 5–6°C.) show little if any increase in the proportion of stained cells. A detailed study of the effect of cold has not been made.

The Effect of Asphyxia.

Suspensions of rat cells were made in previously boiled Locke's solution, and covered with a layer of albolene. These were then placed in the thermostat, and compared with a similar suspension which had been exposed to the air. The asphyxia thus produced gives rise to a notable increase in the percentage of stained cells (Text-fig. 4). A similar effect was brought about by exhausting the oxygen with pyrogallic acid and sodium hydroxide.

If carbon dioxide gas is passed through a suspension of cells, flocculation occurs, the cells sinking in clumps to the bottom and leaving the supernatant fluid clear. The clumps, however, are easily broken up, and if the stream of carbon dioxide is suspended, the emulsion after being shaken resumes its normal appearance. The proportion of stained cells rises slowly, and is higher than that of a control suspension through which air is passed at about the same rate.

Pure oxygen passed through a suspension of cells brings about their rapid destruction. It was first thought that this might be due to trauma caused by the mechanical agitation; but the control in which air is allowed to bubble through vigorously shows that this is not the case.

The Protective Action of Serum and Other Colloids.

The addition of serum, even in relatively dilute amount, greatly lowers the percentage of stained cells, and in sufficient concentra-

[7] Schultze, M., quoted by Marchand, F., in Krehl, L., and Marchand, F., Handbuch der allgemeinen Pathologie, Leipsic, 1908, i, 50.

tion may completely inhibit the staining with the ordinary strength of trypan used. For this reason, in order to bring out differences where sera are being used, it is necessary to add trypan in a concentration of 1: 5,000 or more.

The following experiment is an example of the inhibitory action of serum.

Tube.	Mixture.	Percentage of stained cells.
1	Locke's solution, 0.5 cc. Thymus cells, 3 gtt.	20.6
2	Locke's solution, 0.5 cc. Thymus cells, 3 gtt. Rat serum, 3 "	None.

Various organic and inorganic colloids were tested as to their ability to prevent staining. It was found that gelatin (3 gtt. of a 10 per cent solution) completely inhibited the penetration of the dye. Egg albumin conferred slight protection; starch and gum arabic were without effect in the concentrations tried. Hemoglobin obtained by laking washed cells in distilled water and rendering the solution isotonic with sodium chloride was also found to be inert. A cholesterol emulsion was made by dissolving pure cholesterol in acetone, pouring the solution into distilled water, and removing the acetone by boiling. The opalescent fluid showed no visible particles. After being made isotonic with sodium chloride, a thick suspension of thymus cells was added and the stainability of the cells compared with a suspension in pure salt solution. No protective action on the part of the cholesterol was observed.

The inorganic colloids tested were arsenious sulfide and colloidal iron. The former in stronger concentrations caused agglutination of the cells and was precipitated about them. In weaker dilutions it appeared to act like serum and gelatin in affording protection against staining.

Colloidal iron was precipitated and the cells were agglutinated in all the concentrations tried.

Extraction of serum with chloroform does not affect its protective powers.

The Effect of Certain Photodynamic Substances.

Hematoporphyrin.—The solution contained 1 mg. per 1 cc. dissolved in 95 per cent alcohol.

The toxic effect of this substance, when activated by direct sunlight, is shown in the following experiment.

Tube.	Mixture.	Treatment.	Percentage of stained cells.
1	Locke's solution, 1.0 cc. Thymus cells, 10 gtt.	Sunlight, 1 hr.	19.0
2	0.10 per cent ethyl alcohol in Locke's solution, 1.0 cc. Thymus cells, 10 gtt.	" 1 "	15.6
3	Hematoporphyrin, 1 : 100,000 in Locke's solution, 1.0 cc. Thymus cells, 10 gtt.	Darkness, 1 "	15.0
4	Hematoporphyrin, 1 : 100,000 in Locke's solution, 1.0 cc. Thymus cells, 10 gtt.	Sunlight, 1 "	99+

The cells which had been exposed to the action of hematoporphyrin in direct sunlight, in a dilution of 1 : 100,000[8] showed immediate staining upon the addition of trypan blue. The effect of sunlight alone or of the alcohol is negative, as shown in Tubes 1 and 2.

Chlorophyll.—An impure solution of chlorophyll, obtained by extracting cabbage leaves with 95 per cent ethyl alcohol, and diluted 1 : 100 with Locke's solution, was tested against rat thymus cells as follows:

Tube.	Mixture.	Treatment.	Percentage of stained cells.
1	1 per cent chlorophyll in Locke's solution, 1.0 cc. Thymus cells, 6 gtt.	Darkness, 30 min.	26
2	1 per cent chlorophyll in Locke's solution, 1.0 cc. Thymus cells, 6 gtt.	Arc-light, 30 "	68

Exposure of the serum alone to the action of these photodynamic substances did not impair its subsequent protective power for the cells.

[8] The same solution of hematoporphyrin causes complete hemolysis of washed rat erythrocytes in 1 : 100,000 dilution, the controls kept in the dark remaining unlaked.

*The Effect of Exposure to X-Rays upon the Stainability of the
Lymphocytes.*

Because of the known susceptibility of the thymus cells to x-rays, we tested the value of trypan as an indicator of cell injury by exposing small tubes containing suspensions of cells to radiation.[9] The control tubes were unexposed, and we were not informed until the end of the experiment which of the tubes had been subjected to the x-rays.

No significant differences could be detected as regards the number of stained cells in the tubes which had been irradiated and the controls. In one of the experiments hourly counts were continued for 7 hours, at which time the percentage of stained cells had risen in both tubes so as to make the counts unreliable.

That this seemingly negative result was probably due to the existence of a latent period is indicated by the following experiment.

Experiment 5.—Two healthy young rats of approximately equal weight and age were etherized, and a small portion of thymus was resected. Cell suspensions in Locke's solution were immediately counted with the addition of trypan blue.

Rat 1 gave 5.9 per cent of stained cells.
"   2   "   6.3   "   "   "   "   "

This showed that the thymus cells of the two rats before exposure were almost identical in their reaction to the stain.

3 days later, the wounds being uninfected and the animals apparently in good condition, Rat 2 was given a 15 minute exposure to the x-rays. 24 hours later both animals were killed, and the suspension of thymus cells in Locke's solution was counted in the usual way.

Rat 1 (control) gave 10 per cent of stained cells.
"   2 (x-rayed)   "   23.5   "   "   "   "   "

The difference in the proportion of stained cells, however, does not fully indicate the severity of the injury. The cells of the x-rayed rat were in all stages of fragmentation, and accurate counting was impossible. It was observed that many of the chromatin particles, which made up the bulk of the suspended material, showed great resistance to the staining with trypan. This would indicate that not every form of cell injury is associated with increased permeability to the dyestuff.

We made a number of experiments to determine whether exposure of the serum to the x-ray in any way altered its protective power, and with one unexplained exception we obtained only negative results. It would appear therefore that the inhibitory property of the serum is not affected, at least during the first 6 hours. The possible existence of a latent period has not yet been tested.

A Comparison of the Thymus Lymphocytes in Old and Young Animals.

In view of the involutional changes which occur in the thymus, and less strikingly in the lymphatic tissues generally with advancing age, we compared with this technique the behavior of cells of old and of young animals. The increased resistance of the cells of young animals has been evident throughout the course of the work, but several experiments have been made to test this point specifically.

Experiment 6.—Suspensions were made from the thymus of a young rat and of a full grown adult animal. The cells were centrifuged and washed in two changes of Locke's solution to eliminate any possible action of the blood serum. The stainability of the cells was then compared in the usual way.

|	Percentage of stained cells.		
Time...........................	3.25 to 3.30	3.40 to 3.48	4.35 to 4.42
Young rat......................	11.1	18.2	28.7
Old rat........................	14.4	24.2	36.9

Several similar experiments gave identical results, so that there appears to be no doubt of the increased sensitiveness of the cells of the older animals to the unfavorable environment to which they are subjected under the conditions of the experiment. It will be interesting to extend this comparison to a study of the relative resistance to specific injuries of various kinds.

Fiore and Franchetti[10] in 1914 reported that they had produced premature involutional changes in rats by the injection of serum

[10] Fiore, G., and Franchetti, U., Su di una particolare proprietà del siero di sangue studiata in rapporto all'accrescimento dell'organismo è all'evoluzione del timo, *Atti Accad. med.-fis. fiorent.*, 1914, 87.

Per Cent
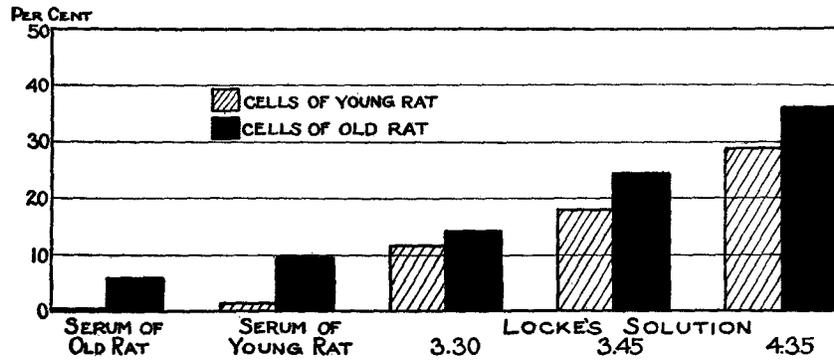
TEXT-FIG. 5. Experiment 6. The comparative stainability of thymus cells of young and of old rats.

Per Cent
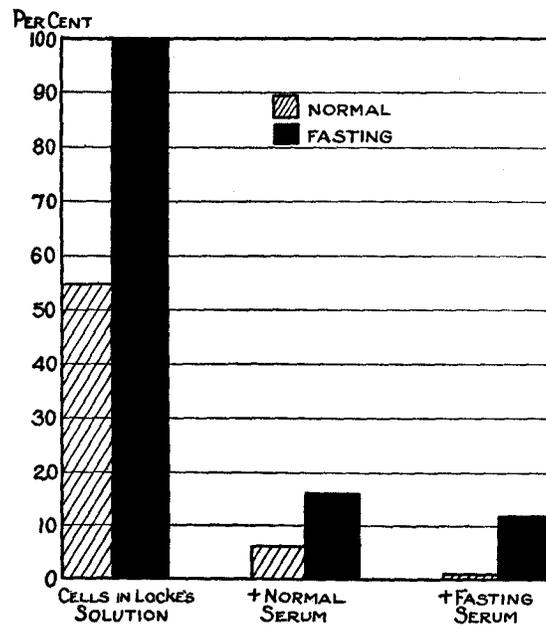
TEXT-FIG. 6. Experiment 7. A comparison of the thymus cells and serum from normal and fasting rats.

from adult animals; and conversely, that the injection of "young" serum into old rats exercised a stimulating effect upon the growth of the thymus. This was contrary to our experience in as far as we had found the plasma of old animals a favorable medium for the growth of thymus tissue *in vitro*. In accord with other workers, we had found that the tissue of young animals showed a more vigorous growth than that of old.

However, the possible toxicity of the serum of old animals as compared with young ones was tested by subjecting young and old cells to the action of young and old serum. The comparative counts made with the usual technique do not indicate a toxic action on the part of the serum from old animals. When equal amounts of either young or old serum are added, there is always an increased stainability of the cells of the old animal. This is shown in Text-fig. 5.

The Effect of Acute and Chronic Inanition upon the Stainability of the Thymus Lymphocytes.

Perhaps the most striking feature in the pathology of the thymus is the atrophy brought about by acute starvation or prolonged inanition. This atrophy is due to the degeneration and partial disappearance of the small thymus cells, and leads, especially in the more protracted cases, to the most extreme reduction in the size and weight of the gland. The hunger involution has been repeatedly studied, both in the human subject, and experimentally by Jonson[11] and by Levin[12] in animals.

The following experiment, which has been performed several times with comparable results indicates that acute starvation brings about a greatly increased stainability of the thymus lymphocytes.

Experiment 7.—Rat 1, weighing 80 gm., was given water only for 3 days, during which period it lost 20 gm. in weight. It was then killed, serum was obtained and a suspension of thymus cells, twice centrifuged and washed, compared with a similar suspension from Rat 2, a control fed daily. Although this control rat

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[11] Jonson, A., Studien über Thymusinvolution; die akzidentelle Involution bei Hunger, *Arch. mikr. Anat.*, 1909, lxxiii, 390.

[12] Levin, S., Recherches expérimentales sur l'involution du thymus, Thèse de Paris, 1912, abstracted in *Zentr. exp. Med.*, 1912, ii, 262.

was severely infected with *Trypanosoma lewisi*, and in consequence ill nourished and anemic, it showed a lower count than the fasting rat (Text-fig. 6).

	Mixture.	Percentage of stained cells.
Rat 1 (fasting)	Locke's solution, 0.5 cc. Thymus cells, 5 gtt.	100
" 2 (control)	Locke's solution, 0.5 cc. Thymus cells, 5 gtt.	54.3

The unusually high count in the control is probably caused by the chronic malnutrition. The average proportion of stained cells in a healthy young rat, as seen by reference to previous experiments, is from 5 to 15 per cent.

The following data exemplify further the regularly noted difference between healthy and ill nourished, diseased animals.

Experiment 8.

Healthy rat.. 8.7 per cent.
Emaciated rat.. 27.0 "   "

The question arose as to whether the serum from a fasting or badly nourished rat contained substances toxic for the thymus cells of a normal animal. It was found, contrary to this idea, that the sera of fasting or emaciated rats conferred a greater protection against staining than did those of the normal controls, possibly because of the greater concentration of the sera under these conditions.

An experiment designed to bring out similar differences in the protective action of sera from normal and marantic infants, upon the stainability of human tonsillar lymphocytes, gave no significant results. The two sera also conferred identical protection upon rat thymus cells.

The Toxic Effect of Heterologous Sera.

That the toxic effect of an alien serum may be manifested in a higher proportion of stained cells is shown in the following experiment, in which the action of rat serum is compared with that of two human sera, upon rat thymus cells.

Experiment 9.

Rat cells—rat serum, 2 gtt................................... 1.4 per cent.
"   " —human serum (A), 2 gtt........................... 9.2 "   "
"   " — "   " (B), 2 " .... 9.4 "   "

The toxicity of normal rabbit and guinea pig serum for rat thymus cells does not appear to be marked, although further studies are necessary. In one experiment the toxicity of horse serum was demonstrated, and also the fact that this toxicity, as judged by the increased percentage of stained cells, was not modified by heating to 56°C. for 15 minutes.

Specific Cytotoxic Serum.

A rabbit which had received four intravenous injections of 2 to 4 cc. of a thick suspension of rat thymus cells, has yielded a serum which is both agglutinative and cytotoxic. The agglutination is evident both micro- and macroscopically. The cytotoxic action is shown in the extreme morphological changes brought about in the cells in suspension (hydropic swelling of cytoplasm, pyknosis, karyolysis, etc.) and also sharply in the increased percentage of cells showing diffuse staining with trypan blue. Inactivation at 58–60°C. completely destroys the toxicity, and the addition of fresh guinea pig complement restores it. The agglutinin is not destroyed at this temperature.

The serum is slightly hemolytic (1 : 10) for rat corpuscles and is also hemagglutinative. The hemolysin can be absorbed from inactivated serum by exposing it to washed thymus cells for 15 minutes at 37°C. On the other hand, exposure of the serum to red blood cells for the same period diminished, but did not wholly remove its thymotoxic action.

Text-figs. 7, 8, and 9 give some of the data upon which these statements are based.

The serum of a rabbit immunized against human tonsil lymphocytes showed a slight toxicity for rat thymus cells as compared with the control normal rabbit serum. This suggests an organ-specific action upon lymphocytes, comparable with that established in the case of spermatoxin and lens precipitin. Further studies are necessary to determine this point. Against human tonsil lymphocytes,
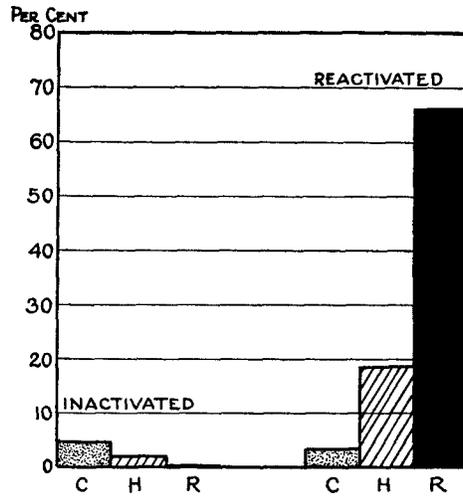
Text-Fig. 7.   Experiment 10.   The effect of thymotoxic serum upon the stainability of rat thymus cells.   C, control serum; H, serum of a rat immunized against human tonsil lymphocytes;   R, serum of a rabbit immunized against rat thymus cells.
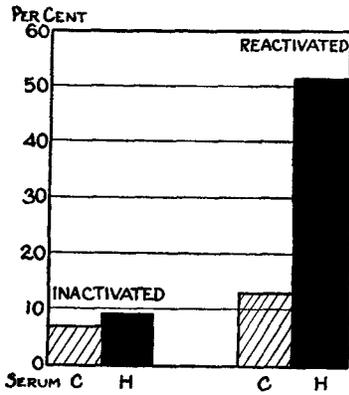
Text-Fig. 8.   Experiment 11.   The effect of lymphocytotoxic serum upon the stainability of human tonsil lymphocytes.   C, control serum; H, serum of a rabbit immunized against human tonsil lymphocytes.
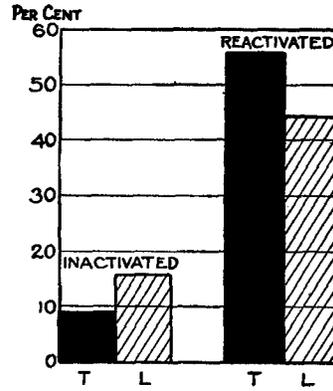
Text-Fig. 9.   Experiment 12.   The action of thymotoxic serum against lymphocytes from the thymus (T) and from a lymph gland (L).

the serum was highly toxic when reactivated with guinea pig complement. This is shown in Text-fig. 8.

Because of the discussion which has raged for many years over the question of the identity of the small thymus cells with the lymphocytes of other tissues, we undertook to test by this method the action of the thymotoxic serum upon the lymphocytes obtained from lymph glands.

Text-fig. 9 shows that both the agglutinative and cytotoxic actions are exerted equally upon lymphocytes of thymic and lymph gland origin. This seems to be strong evidence of their biological identity, more convincing perhaps than inferences based upon a common morphology.

CONCLUSIONS.

A simple method is presented by which, with the diffusion of trypan blue into the nucleus as a criterion of cell injury, it is possible to study quantitatively the effect of various agencies upon the small thymus cells and upon the tissue lymphocytes.

Preliminary studies with this method have led us to the following conclusions, which, however, unless otherwise stated, may be taken as applying only to the lymphocytes of the rat thymus.

1. The small thymus cells, when suspended in balanced phosphate solutions, show no distinct reaction to variations in hydrogen ion concentrations ranging between P_H 7.0 and P_H 7.8. Beyond P_H 7.0 there is a sudden increase in the permeability of the cells to the dye; plasmolysis of the cells occurs when the alkalinity exceeds P_H 8.0.

2. Heating to 49° or 50°C. is accompanied by a critical increase in the permeability of the cells to the dye.

3. The injury caused by lack of oxygen can be demonstrated by the increase in the number of stained cells.

4. The addition of serum to suspensions of thymus cells or tonsil lymphocytes greatly inhibits the diffusion of the trypan into the cells. The protection afforded is roughly proportionate to the amount of serum added.

Gelatin also exerts a marked protective influence; egg albumin affords a partial protection; starch and gum arabic are inert. Hemoglobin and cholesterol do not modify the stainability of the cells.

Arsenious sulfide in weak concentrations partially inhibits the diffusion of the dye.   Colloidal iron is without effect, and is precipitated about the cells.

5. The toxicity of the photodynamic substance, hematoporphyrin, and of an impure chlorophyll solution in the presence of sunlight could be strikingly demonstrated by the greatly increased permeability of the cells to the stain.

6. Acute and chronic inanition produces an increased fragility of the cells.   The protective power of the serum in acute starvation appears to be increased.

7. The small thymus cells of old animals are more readily injured than are those of young ones, as indicated by the increased proportion of stained cells.

8. The method has been applied to the demonstration of the action of cytotoxic immune sera for rat thymus cells and for human tonsil lymphocytes *in vitro*.   Further experiments dealing with the question of specificity are in progress.   The cytotoxins are inactivated by the addition of complement.   Thermostabile cytagglutinins have also been produced.

I wish in conclusion to express my obligations to Miss Kate Brogan for her assistance in the technical part of the work.