due (1) to a growth through the vessels, *i.e.*, intracytoplasmic multiplication of the parasites and ultimate distintegration of the cells constituting their walls, with secondary involvement of the parenchymal cells by the parasites, and (2) to thrombosis of involved blood vessels with secondary necrosis of parenchymal cells without preliminary parasitization. The kidneys were again, strangely enough, only irregularly affected, and even then only the interstitial vessels seemed to suffer while the glomeruli and tubules appeared almost uninvolved. The destruction of cells either directly by the parasites or indirectly by thrombosis of blood vessels whose walls were parasitized comprised the outstanding pathological changes, while inflammation was either absent or, in certain instances, a late manifestation.

Intracerebral inoculation in *rhesus* monkeys was followed only by a febrile disease. Intracutaneous injection gave rise to a local lesion associated, as after other forms of peripheral inoculation, with systemic disease and the presence of the parasites in the circulating blood, as demonstrated by mouse inoculation.

The toxoplasma also appear to offer an opportunity for direct investigation of certain as yet obscure problems in immunity of obligate intracellular parasites. It has been possible to show, for example, that *rhesus* monkeys recovering from an infection with toxoplasma are immune to reinoculation and that the serum of such monkeys contains antibodies which may be termed "neutralizing" or "protective." The "neutralization" or "protection" tests were performed in the same manner as with viruses, *i.e.*, by mixing *in vitro* the serum with a tissue suspension or exudate containing the parasites and injecting the mixture intracerebrally or intraperitoneally in mice or intracutaneously in rabbits. The latter proved to be the method of choice, since the parasite suspension as well as a number of different sera could all be titrated quantitatively on the back of one rabbit. It was interesting, however, that rabbits which recovered from the non-fatal disease induced by the intracutaneous injection of the "unchanged" toxoplasma developed a solid tissue immunity resisting the constantly fatal intracerebral injection of the same strain, as well as inoculations with the highly pathogenic and fatal changed strain, but, as a rule, had no demonstrable protective humoral antibodies. In some rabbits only sufficient antibody to protect against a single skin infective dose was present. Similar observations are not uncommon with certain viruses.

Preliminary studies on the protective antibody in convalescent monkey sera revealed that it apparently had no effect on the toxoplasma *in vitro*. No agglutination or disintegration of the parasites could be observed in mixtures which proved innocuous on animal inoculation. Centrifugation of such mixtures after incubation for several hours *in vitro* and separation of the parasites from the serum showed that they had retained their infectivity. Further studies now in progress on the nature of this protective antibody, as well as the solid tissue immunity unassociated with such antibodies, are expected to yield data of interest to the understanding of similar phenomena with other obligate intracellular parasites.

The rabbit skin protection test may, perhaps, also prove useful in the diagnosis of infection with toxoplasma. The present evidence that they cause disease in man is rather tenuous and has been questioned by many competent parasitologists. The reason for this uncertainty is that the diagnosis has been based either entirely on morphological grounds without tests for pathogenicity or only on animal inoculation. In a recent study[3] on glandular fever (infectious mononucleosis) doubt arose as to whether the toxoplasma which were isolated were derived from the patients' blood or from the experimental animals (rabbits) which might have been spontaneously infected. The protection test just described might aid in elucidating this problem.

The work just outlined will be described in detail in a future communication. The aim of the present report is primarily to call attention to the existence of toxoplasma in North America and to point out their obligate intracellular parasitism, a study of which reveals many features in common with certain of the filtrable viruses, particularly as regards pathogenesis, cultivation, immunity and other host-parasite relationships.

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## HEPARIN AND THE FORMATION OF WHITE THROMBI

IN a recent preliminary communication Murray, Jaques, Perrett and Best[1] reported that the incidence of thrombus formation after mechanical or chemical injury to veins was appreciably decreased when a solution of purified heparin was administered to the dogs before and for adequate periods after the injury. In animals which did not receive heparin the injury was followed by the appearance of typical thrombi. Areas exhibiting the structure of thrombi could be observed in many of the histological sections made through the obstructing mass. Since the veins from the heparinized animals were in many cases free of obstruction,

[3] J. O. W. Bland, *Lancet*, 2: 521, 1930; *Brit. Jour. Exp. Path.*, 12: 311, 1931.

[1] D. W. G. Murray, L. B. Jaques, T. S. Perrett and C. H. Best, *Can. Med. Assoc. Jour.*, 35: 621, 1936.

it would appear that adequate amounts of heparin are effective in preventing the accumulation of platelet masses on the injured surfaces of veins.

The opinion has been wide-spread, however, probably largely as a result of a publication of Shionoya, that while heparin prevents the clotting process it does not influence the deposition of platelets. Rowntree and Shionoya[2] studied the accumulation of platelets in an "extracorporeal loop." Using this technique, Shionoya[3] reported that heparin had no influence upon the rate of formation of white thrombi.

We have repeated the experiments of Shionoya and find that in dogs when the observations are made over a two- or three-hour period, there is almost invariably formation of white thrombi in the glass cannulae and the collodion or Cellophane tube used to connect the arterial with the venous cannula. Masses of white thrombi, large enough to produce partial or complete obstruction of the tubes, have been observed in 15 of the 16 experiments in which no heparin was used. In experiments, however, in which one intravenous injection of a solution of purified heparin (450 units per kilogram) was made, we have never observed any obstruction of blood flow or the accumulation of any platelet masses.

Microscopic examination of the obstructing masses in the experiments in which heparin was not used revealed, in every case, the typical structure of the white thrombus. In all except two of the experiments in which heparin was used microscopic examination of the Cellophane loop revealed only a few red and white blood cells. In two cases *very minute patches* of material which appeared to be composed of platelets were seen. It may be stated, however, that in no case was any mass which had the characteristic structure of a thrombus observed in the experiments in which heparin was administered.

The results of these studies demonstrate that purified heparin is very effective in preventing the formation of white thrombi under the conditions of our experiments.

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## LIGHT AND THE SEXUAL CYCLE OF GAME BIRDS

SINCE Rowan's[1] original observations that lengthening the period of light in winter by means of artificial illumination caused stimulation of normally quiescent gonads in juncoes, similar results have been found in other species of birds and some mammals.

As a result of night lighting of upland game birds, Petty[2] secured gonad stimulation and eggs in quail during the non-breeding season in 1934, Martin[3] announced mid-winter production of eggs in pheasants (*Phasianus colchicus torquatus*) in 1935, Clark, Leonard and Bump[4] secured gonad stimulation in pheasants, quail and grouse (*Bonasa umbellus*) and Bissonnette and Csech[5] secured early eggs from pheasants and quail by night lighting.

We wish to report briefly some of our experiments concerning the factors regulating sexual activity in the pheasant and grouse.

(1) The absence of light inhibits the onset of sexual activity in the grouse and pheasant. Two pair of grouse were continuously in the dark, except for a low illumination of 0.02 foot candles over the feeder from February 10 to June 20, 1936. No sexual activity was manifested during this time, although the experiment extended over the period of sexual activity of the control birds. Two pair of pheasants under the same experimental conditions also failed to come into breeding.

(2) Continuous illumination during the winter months can stimulate grouse into sexual activity and egg laying but does not prevent the cessation of egg laying. A pair of grouse were illuminated continuously, starting on February 10, by two 150 watt lamps. No daylight was admitted and the light intensity was maintained constantly at 22 foot candles. Egg laying began on March 6 and continued to April 3, yielding 14 eggs, the normal number for grouse. None of the control grouse laid before April 10. The experimental birds ceased laying in spite of the continued light. We were able to secure egg production prematurely in pheasants and quail with night light, but did not carry the experiment to the end of the normal breeding season.

(3) The cessation of egg laying is probably due to a failure of the hypophysis to furnish the necessary gonad stimulating hormones rather than to an exhaustion of the gonads. On June 23, 1936, two pair of grouse which had completed their reproductive cycle were autopsied, and it was found that the sex organs had regressed to approximately the mid-winter condition. The testes of the males weighed 41.4 and 41.6

[2] L. G. Rowntree and T. Shionoya, *Jour. Exper. Med.*, 46: 7, 1927.
[3] T. Shionoya, *Jour. Exper. Med.*, 46: 19, 1927.
[1] W. Rowan, *Nature* (London), 115: 494–495, 1925.
[2] *American Field*, August 11, 1934.
[3] L. E. Martin, *Game Breeder and Sportsman*, 39: 95, April, 1935.
[4] L. B. Clark, S. L. Leonard and G. Bump, SCIENCE, 83: 2150, 268, March 13, 1936.
[5] T. H. Bissonnette and A. G. Csech, SCIENCE, 83: 2156, 392, April 24, 1936.