modified Thomas[2] procedure proposed by Mitchell[3] are now under way. Similar feeding experiments with isolated cereal proteins which have been heat treated, and with water-extracted cereals to eliminate the effect of possible caramel-like bodies are also under way.

2972

The nature of immunity to a protozoan infection.

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In malaria infections it is a common observation that some people are more resistant than others, an observation confirmed experimentally by Celli in 1901[1] and more recently by Yorke and Macfie,[2] who showed that there are some individuals who are resistant to experimental infection.

An experimental study of this problem with malaria parasites was out of the question until the advent of the treatment of paresis by a superimposed malaria infection. We, therefore, decided to study the problem in the case of another protozoan infection, the trypanosome, which produced in experimental animals a relapsing, fatal, disease.

Our studies thus far have brought out a number of facts bearing on this problem:

(1) *Acquired immunity after cure.* None of the animals, rabbits or guinea pigs, used in our experiments, and the number is now fairly large, failed to become infected, or recovered spontaneously from the infection. However, animals cured of the infection with Bayer 205 acquire a resistance to reinfection quite distinct from the possible protective action due to the drug itself. These experiments have been repeated many times, with both rabbits and guinea pigs and the result is quite constant. Infected

[2] Thomas, K., *Arch. Physiol.*, 1909, 219.

[3] Mitchell, H. H., *J. Biol. Chem.*, 1924, lviii, 873.

[1] Celli, A., *Die Malaria*, 1913.

[2] Yorke, W., and Macfie, T. W. S., *Trans. Soc. Trop. Med. and Hyg.*, 1924, xviii, 13.

animals treated and cured with minimal amounts of Bayer 205 (0.05 gm. per kilo), which in control animals gave only temporary protection,[3] became refractory to repeated attempts at reinfection for at least three to five months. Kleine and Fisher,[4] working with monkeys, also found that the cured animals were more resistant to reinfection than normal animals receiving the same dose of the drug.

This is, therefore, an experimental demonstration of an acquired immunity to a protozoan infection. Similar results have recently been reported by Chesney and Kemp,[5] and by Voeigtlin and Dyer,[6] in experimental treponema infections in rabbits cured with Salvarsan or other drugs. Apparently animals cured from a protozoan infection manifest an acquired resistance to reinfection.

These results opened two lines of investigation. On the one hand we studied the possibility of artificial immunization, and on the other we tried to ascertain the mechanism of the acquired resistance.

Artificial immunization to trypanosome infection. Attempts to immunize rabbits against trypanosomes by the usual methods of repeated infection of the organisms yielded most unexpected results. The trypanosome material was obtained from heavily infected guinea pigs, by centrifugation and washing. Whatever method we employed we obtained increased sensitization to infection instead of increased resistance. In one series of experiments two sets of rabbits were treated with disintegrated trypanosomes, obtained by repeated freezing and thawing. The animals received three injections at intervals of five days. Other sets were inoculated over long periods (ten, twenty and twenty-five injections), with whole trypanosomes. But whatever the method of treatment, the animals became so sensitive that the incubation period after the infection was about one fourth or less than the usual interval. The blood infection during the first few days was heavy and many of the animals showed swelling of the eyelids and ears, with the onset of the infection.

Since the immunity observed in cured animals developed after treatment with a drug (Bayer 205), we repeated the immuniza-

[3] Kligler, I. J., and Weizman, I,. *Ann. Trop. Med.*, 1924, xviii, 437.

[4] Kleine, K. F., and Fisher, W., *Deutch. Med. Wchnschr*, 1922, xlviii, 1693.

[5] Chesney and Kemp, *J. Exp. Med.*, 1924, xxxix, 553.

[6] Voegtlin, C., and Dyer, H. A., *Pub. Health Rep.*, 1925, xl, 2511.

tion experiments, using mixtures of Bayer 205 and trypanosomes. The trypanosomes were obtained from infected guinea pigs, as before, and mixed with amounts of the drug (0.005 gm. per kilo), known from previous experiments not to give any protection against infection.

This procedure gave totally different results than those reported above. The animals treated with the mixture developed a degree of resistance, in all of the experiments, quite distinct from that conferred by the drug alone. In one experiment the treated animal never developed the infection, and in the others the incubation period was greatly prolonged, usually about twice as long as that in the control animals treated with the drug alone. Why the injection of trypanosomes alone sensitizes the animals while trypanosomes mixed with Bayer 205 increases their resistance is a problem for further investigation.

Mechanism of resistance. The determination of the nature of the resistance presented certain difficulties. The species of trypanosome used in the experiments, *Tr. evansi,* produces a chronic, relapsing infection which is always fatal. This implies that there is a native resistance slowly broken down by the parasite. It seemed likely that the acquired and native resistances are in some way related, but their nature was not clear. Attempts to identify humoral antibodies *in vitro* tests, and by the Pfeifer phenomenon in guinea pigs, gave negative results. Recently Luengo and de Buen[7] claimed to have demonstrated specific lytic substances in the blood serum of a patient cured with Bayer 205, using the mouse as the test animal. These results were confirmed by Kiang[8] in experimental animals, also using mice as the test animals.

Our experiments indicate that the natural and acquired resistances are closely related to the form elements of the blood. Infected animals treated with large doses of olive oil (5 to 10 cc.) relapse very promptly, usually in 24 to 48 hours after the injection. Similarly cured animals, receiving an injection of a large dose of oil followed by an infective dose of trypanosomes, develop an infection, when other animals cured at the same time and reinfected with the same dose still resist the infection. Finally, animals in which the leucocytes have been destroyed by the in-

[7] Luengo, E., and De Buen, S., *C. R. Soc. Biol.*, 1924, xci, 825.

[8] Kiang, T. S., *Arch. f. Schiffs u. Tropen. Hyg.*, 1925, xxix, 572.

jection of benzol develop a blood infection after an incubation period of one day, or about one fourth to one sixth the usual time.

These experiments indicate that the mechanism of resistance resides to a large extent, if not wholly, in the form elements of the blood, or, as it is usually designated, the reticulo-endothelial system. Both the oil and the benzol have some effect on the large endothelial cells. Olive oil apparently renders them ineffective by blocking them. An examination of the peritoneal fluid during the first few days after its injection shows a very large increase in the large mononuclear cells, in a state of great activity, many of them filled with the oil. It is probably this blocking that results in a lowered resistance of the animal to the invasion of the parasite. After recovery, a week or so after the injection, the resistance of the animal is apt to be greatly increased. Benzol produces the same effect in a different manner. Injection of benzol causes a primary leucocytosis, followed by a rapid destruction of the leucocytes. During this period the resistance of the animal is greatly decreased, so that the multiplication of the parasites proceeds rapidly and the blood is promptly invaded. On recovery the resistance of these animals may be even greater than that of normal animals, and blood invasion is more uncommon than in normal controls.

Summary. These experiments show: (1) That trypanosome infected animals cured with Bayer 205 develop acquired resistance to reinfection lasting several months. (2) That repeated injections of trypanosomes causes hypersensitization. (3) That injection of a mixture of trypanosomes and Bayer 205, leads to partial immunization. (4) That the resistance, both natural and acquired is due in large measure to endothelial cells, because blocking with olive oil or destruction of the leucocytes with benzol breaks down the resistance.