Results. No significant deviation from the normal healing of stomach wounds was discovered in young rats maintained upon a ration deficient in cystine and methionine. The curves of healing of these wounds, as measured by their tensile strengths, were practically identical in the control and in the experimental groups. (Graph I.) (The distended stomachs ruptured at places elsewhere than the operative sites in 10 of the 11 control animals, and in 15 of the 17 experimental animals for the 8-, 10- and 12-day periods.) These findings do not warrant any opinion concerning the allegedly essential rôle of sulphydryl compounds as the real stimuli to cell growth and proliferation. Nor can any conclusions be justly drawn regarding the absolute indispensability of the sulphur-bearing amino-acids in the process of fibroblastic proliferation and wound healing. If these substances are indeed essential in this process, then it must be postulated that the animal, not provided with enough of the sulphur compounds in its diet to allow adequate body growth, is still able to derive from some other source a quantity sufficient to permit adequate wound healing. This source is possibly endogenous and may be related to the breakdown of its own body proteins.

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Biological and Immunological Identity of Toxoplasma of Animal and Human Origin.

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There exists considerable confusion among parasitologists as to the characteristics which justify the classification of Protozoa of certain morphology as *Toxoplasma.* The capacity to multiply and to produce disease in a variety of hosts, including mammals and birds, must, in accord with the conclusions of Aragao,[1] be regarded as the chief taxonomic characteristics of the group. Morphology as the only guide can be misleading and confusing as is evident from the present controversy as to whether certain forms observed in avian malaria are *Toxoplasma* or stages of *Plasmodium.*[2] In accord with Aragao's criteria, the existence of *Toxoplasma* in North America was first demonstrated by Sabin and Olitsky,[3] who isolated *Toxo-*

1 Aragao, H. de B., *Compt. rend. Soc. biol.*, 1933, **113**, 214.

2 Hegner, R., and Wolfson, F., *Am. J. Hyg.*, 1938, **27**, 212; *ibid.*, 1938, **28**, 437.

3 Sabin, A. B., and Olitsky, P. K., *Science*, 1937, **85**, 336.

plasma from the brain of a guinea pig and showed them to be pathogenic for guinea pigs, rabbits, mice, monkeys and chickens. The forms seen by Mooser[4] in guinea pigs in Mexico and by Markham[5] in the United States may have been *Toxoplasma* but in the absence of transmission-experiments, the diagnosis remained uncertain. The reports by Manwell and Herman,[6] Herman,[7] and Wood and Wood[8] of the presence of *Toxoplasma* in North American birds cannot be accepted as proved, since the identification was made only on morphologic grounds, while transmission to other birds was unsuccessful and no tests were made on mammals. The parasites identified by Wolfson[9] as *Toxoplasma* in canaries were transmitted to other canaries but no tests with mammals were reported. Similarly it may be said that while human infection with *Toxoplasma* has been suggested by several investigators on morphological grounds, it has not hitherto been proved by adequate animal transmission and identification. The circumstances under which Bland[10] obtained *Toxoplasma* in a rabbit after inoculation with blood from a patient suffering from glandular fever (infectious mononucleosis) were such that one could not be certain whether they originated in the human blood or the rabbit. In view of the demonstration that monkeys recovering from experimental toxoplasmosis developed neutralizing antibodies for the parasites,[3] a number of sera from patients recovered from infectious mononucleosis were tested for such antibodies against our *Toxoplasma* and none was found.

In my opinion the first definite evidence that *Toxoplasma* can infect human beings has just been supplied by Wolf, Cowen, and Paige[11] with a case of encephalitis in a child. They not only demonstrated parasites of typical morphology in the human tissues but isolated *Toxoplasma* from a large number of rabbits and mice that were injected with the human brain. The fact that they used a large number of animals for transmission and that so many of them developed the infection almost simultaneously after a suitable incubation period leaves little doubt that the parasites originated in the human tissue. After several passages in mice these investigators submitted to this laboratory for a comparative study their *Toxo-*

4 Mooser, H., *J. Infect. Dis.*, 1929, **44**, 186.

5 Markham, F. S., *Am. J. Hyg.*, 1937, **26**, 193.

6 Manwell, R. D., and Herman, C. M., *J. Parasitol.*, 1935, **21**, 415.

7 Herman, C. M., *Am. J. Hyg.*, 1937, **25**, 303; *Tr. Am. Micr. Soc.*, 1938, **57**, 132.

8 Wood, F. D., and Wood, S. F., *J. Parasitol.*, 1937, **23**, 197.

9 Wolfson, F., *J. Parasitol.*, 1937, **23**, 553.

10 Bland, J. O. W., *Lancet*, 1930, **2**, 521; *Brit. J. Exp. Path.*, 1931, **12**, 311.

11 Wolf, A., Cowen, D., and Paige, B., *Science*, 1939, **89**, 226.

plasma of human origin in the form of an infected mouse. As regards pathogenicity for a wide host-range, including mammals and birds, the *Toxoplasma* of human (Hum.) origin corresponded in every respect to those of animal (An.) origin. Mice injected intracerebrally (0.03 cc) and intraäbdominally (0.5 cc) with infected mouse-brain died with nervous signs after incubation periods of 5 to 8 days. When the inoculation was made only intraäbdominally, all mice became sick, many with nervous signs, and the majority died while some survived with chronic disease and infection. The parasites seen in films of the peritoneal exudate, the brain, and viscera were morphologically identical with the ones studied in this laboratory for the past 4 years. Intracutaneous injection of 0.2 cc to 0.3 cc of infected mouse-brain suspension on the back of rabbits, was followed by the development (after 3 to 4 days) of a characteristic indurated skin-lesion, the center of which eventually underwent hemorrhagic necrosis, and of a cycle of fever of 5 to 8 days' duration, terminated by either death or recovery; of four rabbits inoculated in this manner, 2 died and 2 recovered. Not only were the cutaneous lesions and the clinical course indistinguishable from those induced by the "An." *Toxoplasma,* but pathologically there was also a striking similarity in the presence of necrotic foci in the viscera, especially the liver.

Two 1-day-old chicks (Rhode Island Reds) inoculated intracerebrally with 0.06 cc of a 10% suspension of infected mouse-brain developed nervous signs on the 5th and 6th days respectively. One died on the 6th day and the other was sacrificed when *in extremis. Toxoplasma* were demonstrated in stained impression-films of their brains and by passage to other chicks. Of two 3-weeks-old chicks inoculated intracerebrally with 0.1 cc of the same mouse-brain suspension, one exhibited transitory weakness and very slight incoordination on the 5th and 6th days, while the other showed no signs of disease; at the end of 4 weeks *Toxoplasma* were demonstrated in the brains of both these chicks by combined intracerebral and intraäbdominal inoculation of mice. Passage to other chicks was possible when the infected chick brain was injected intracerebrally but not intramuscularly (Table I).

The immunological identity of the *Toxoplasma* of animal and human origin was established by active cross-immunity and by neutralization-tests. Two rabbits that had recovered from an intracutaneous inoculation of "An." *Toxoplasma* were injected intracutaneously with "Hum." *Toxoplasma* along with 2 normal controls. The 2 convalescents remained well without developing either the typical skin-lesion or fever, while the controls contracted the charac-

TABLE I.
Pathogenicity of *Toxoplasma* of Human Origin for Chicks.

Experiment	Source of *Toxoplasma*	Route of inoculation	Dose, cc	Age of chicks	Chick No.	Result	Remarks
A	Mouse-brain suspension*	Intracerebral	.06	1 day	1	CNS 5th, dead 6th	*Toxoplasma* present in film of brain
					2	CNS 6th, sacrificed 6th	" " " " " " Brain-passaged to other chicks
			.10	3 wk	3	Slight CNS 5th, 6th; recovered	*Toxoplasma* in brain 4 weeks after inoculation demonstrated by mouse-passage
					4	No signs of illness	*Toxoplasma* in brain 4 weeks after inoculation demonstrated by mouse-passage
B	Chick-brain suspension	Intracerebral	.06	5 days	5	CNS 6th, dead 7th	*Toxoplasma* present in film of brain
					6	Slight CNS 7th, 8th; recovered	*Toxoplasma* in brain 4 weeks after inoculation demonstrated by mouse-passage
		Intramuscular	1.00	5 "	7	No signs of illness	Brains, lungs, and spleens injected in mice 1 month after inoculation; no *Toxoplasma* obtained
					8	" " " "	
					9	" " " "	

*Four mice inoculated intracerebrally with 0.03 cc of same suspension exhibited signs of encephalitis on 5th day and were dead on the 6th.

CNS 5th = Signs of encephalitis on 5th day.

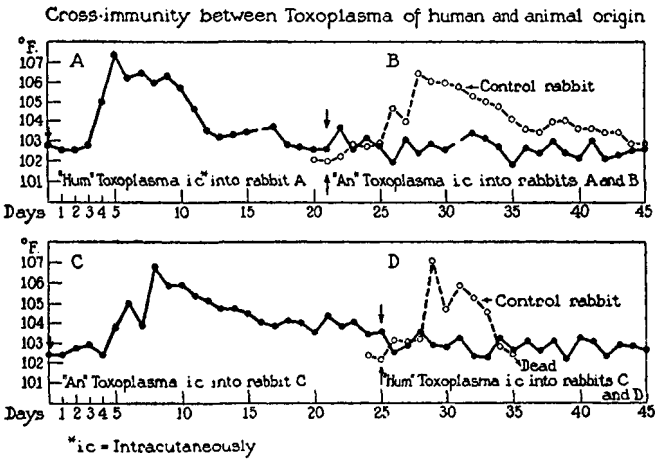
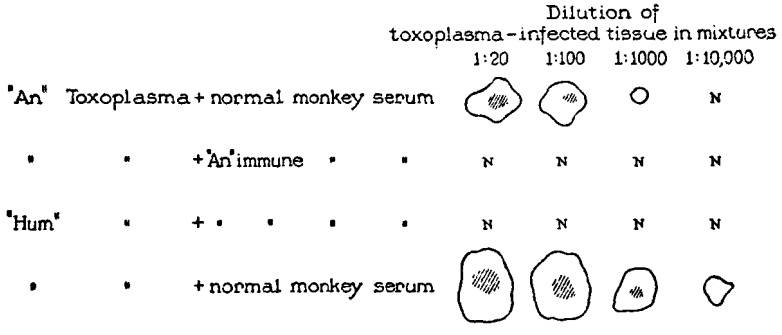


CHART I.

teristic disease. The same result was obtained when 2 "Hum." *Toxoplasma* convalescents were reinoculated with "An." *Toxoplasma* along with 2 controls. Chart 1 shows the temperatures of one set of rabbits in each test. The neutralization-test was carried out with a hyperimmune monkey's serum prepared against the "An." *Toxoplasma* in 1935. A 10% suspension in Tyrode's solution of infected mouse-brain suspension was allowed to sediment spontaneously for a half hour; the supernate and dilutions prepared from it in Tyrode's solution were mixed with equal amounts of undiluted immune or normal monkey's serum. After 10 minutes at room-temperature

Neutralization of Toxoplasma of human and animal origin by serum of monkeys hyperimmunized with "Animal" Toxoplasma



Mixtures injected intracutaneously on the back of a single rabbit. Resulting lesions traced on 12th day

////// = Necrosis N = No lesion

CHART 2.

0.2 cc of each mixture was injected intracutaneously on the back of a single rabbit. The results are shown in Chart 2.

The remarkable immunological and biological identity between the *Toxoplasma* of animal origin and the first strain of human origin suggests that the same protozoön may operate in all susceptible mammals, a fact which must be considered in the epidemiology of toxoplasmosis. The incidence of toxoplasmosis in animals and human beings remains to be determined, and the existence of clinically inapparent or unrecognized non-fatal cases will very likely be found to play a definite rôle in the dissemination of the infection. These studies also suggest that unless the parasites of birds which resemble *Toxoplasma* morphologically, but are not pathogenic for or do not multiply in mammals, can be shown to possess some immunological relationship to the classical *Toxoplasma,* they should be included in a separate group.

Conclusions. *Toxoplasma* of animal and human origin have been shown to be identical biologically in their pathogenicity for mammals and birds, and immunologically by producing an active immunity against one another and by the fact that a serum against one neutralizes both.

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Hypercalcification, -Calcemia and -Lipemia in Chickens Following Administration of Estrogens.

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Previous studies have shown that pigeons treated with estrogenic substances show a decided rise in blood calcium and a replacement of the marrow cavity by newly-formed endosteal bone.[1] It seemed of interest, therefore, to study the effect of estrogen on the bones and on the blood calcium of the domestic fowl. While our experiments were in progress, Zondek reported that estrogens increase bone calcification

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1 Pfeiffer, C. A., and Gardner, W. U., *Endocrinology,* 1938, **23**, 485.