the primary factor since in the acute starvation subjects this was at a very high level whereas in the semi-starvation subjects it was sharply reduced.

There is the possibility that the decrease in the ketosteroid excretion may be explained on the basis of a change in the activity of the testes. During the semi-starvation experiment there was a reduction in sexual functions as revealed by alteration in libido and in the morphology and physiological responses of the sperm collected at that time. Similar studies were not made in the acute starvation experiment, but here, too, at least libido was decreased. Other factors than the testes may be considered in explaining the decrease in ketosteroid excretion observed in acute and semi-starvation. This is apparent when one considers the marked differences in the ketosteroid excretion in these two experiments. Changes in the function and activity of the adrenals under these conditions can only be surmised at the present time.

The hard physical work in the acute starvation experiment had no influence on the ketosteroid excretion since the same work was done in the control period. Furthermore, in other experiments where the same work was done while the men were in caloric balance, there was no change in the ketosteroid excretion. Besides the physical "stress" in the acute starvation, both experiments were associated with considerable mental "stress" but in spite of this the ketosteroid excretion in each experiment decreased. This is contrary to other reports in the literature which have emphasized the increased ketosteroid excretion in "stressful" situations.[8]

Summary. The urinary excretion of total, neutral 17-ketosteroids was determined in 10 young men who, over a 6-months period, lost 24% of their original body weight. The reduction in ketosteroid excretion was 30% of the control values.

Twelve young men performed hard physical work during 4 days when they received no food. By the fourth day the urinary excretion of 17-ketosteroids was only 31% of the control value. The ketosteroid excretion was not influenced by the hard work since the subjects were trained to it before the start of the fast.

8 Pincus, G., and Hoagland, H., *J. Aviation Med.*, 1943, **14**, 173; Hoagland, H., *Science*, 1944, **100**, 63; Pincus, G., in *Recent Progress in Hormone Research*, Acad. Press, Inc., New York, 1947.

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Isolation of Toxoplasma from Cerebrospinal Fluid of a Living Infant in Holland.

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(Introduced by Albert B. Sabin.)

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Recent observations raise the supposition that toxoplasmosis is not infrequent in Europe. In most of the cases, the parasitological confirmation of the clinical diagnosis has been made post mortem by the demonstration of toxoplasma in brain sections. Toxoplasma have been demonstrated rarely in the cerebrospinal fluid during life of the patient by microscopic examination and animal inoculation. In the summer of 1947, however, we succeeded in this.

Our patient, the second child of apparently healthy parents, was a 6-weeks-old boy who did not show any signs of illness during the first few weeks of life. On August 7, the infant was admitted to the hospital, showing

TABLE I.
Toxoplasma Neutralization Tests in Rabbits.

Rabbit No.	Toxoplasma strain	Toxoplasma mixed with	Dilution of mouse brain suspension 1:10	1:50	1:500	1:5000
203	Bk	Tyrode's sol.	++++	+++	+	—
	Bk	Mother's serum	+	+	—	—
239	S	Tyrode's sol.	++	+	+	—
	S	Mother's serum	+	—	—	—
232	Bk	Tyrode's sol.	++++	++++	++	—
	Bk	Immune monkey serum	++	+	—	—
	S	Tyrode's sol.	++++	++++	+	—
	S	Immune monkey serum	++	+	—	—

++++ Strong swelling and necrosis.
+++ Strong swelling without necrosis.
++ Moderate swelling without necrosis.
+ Slight swelling.
— No reaction.

TABLE II.
Cross Immunity Between "Bk" and "S" Strains of Toxoplasma.

Rabbit No.	First inoculation Date	Strain	Reaction	Second inoculation Date	Strain	Reaction	Strain	Reaction
250	Oct. 14	S	++++	Dec. 1	S	+	Bk	+
202	Nov. 12	S	++++	1	S	++	Bk	++
213	7	Bk	++++	1	S	++	Bk	+
214	7	Bk	++++	1	S	++	Bk	++

Significance of + to ++++ as in Table I.

twitchings of arms and legs, rotations of the eye-balls, developing hydrocephalus, neck stiffness, opisthotonos, muscle rigidity and scissor stand of the legs; soon convulsions appeared.

Dr. C. D. Binkhorst, who examined the eyes, diagnosed anterior chorioretinitis, bilateral peripheral ablatio retinae, atrophy of the papillae and strabismus convergens. Dr. Berkyens, who performed a ventriculography, reported displacement and enlargement of the lateral ventricles, especially of the right one which was displaced to the left, ½ inch beyond the median line.

The cerebrospinal fluid, obtained by ventricular puncture, contained xanthochrome and tryptophane. The reactions of Nonne and Pandy were strongly positive, and there were 196 cells per cu mm. In the smear of the centrifuged sediment of this fluid (August 7) we found many halfmoon-shaped parasites resembling toxoplasma. They were not found in the blood, urine and bone-marrow.

On August 11, fresh cerebrospinal fluid was inoculated intracerebrally into 10 mice; 8 of them died after 7 to 25 (average 15) days, and toxoplasma could be demonstrated in the brains. In serial transmissions, the length of the incubation period decreased and from the third intracerebral passage 100% of the mice died after 5 to 6 days.

Guinea pigs inoculated intracerebrally with fresh cerebrospinal fluid from the patient, remained healthy, but those inoculated with toxoplasma-containing mouse brain suspensions died after 12 to 15 days.

Dr. A. B. Sabin kindly provided us with a toxoplasma strain of human origin (called by us strain S) for comparison with ours (strain Bk). We carried out a number of neutralization experiments (rabbit skin tests as described by Sabin[1]) with both strains, which were tested against serum of the infant's mother and against lyophilized toxoplasma immune monkey serum. The latter

[1] Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 6.

we also received by courtesy of Dr. Sabin. The results of the rabbit skin tests are recorded in Table I, from which it becomes evident that both of the sera neutralized the strains equally (between 10 and 50 skin test doses). The same was true with sera from a group of children suspected of having toxoplasmosis and with sera from their mothers; both of the strains were neutralized to the same titer.

Of 16 rabbits used for the skin test, 8 died 9 to 12 days after the inoculation. At autopsy an enlarged spleen with white foci, containing toxoplasma, was found regularly. Of the 8 surviving animals 4 were re-inoculated intracutaneously with 0.2 cc of a 10% mouse brain suspension at 4 separate spots, in order to test cross-immunity. Skin lesions of moderate size developed, but without necrosis. The results of the cross-immunity experiments are shown in Table II.

There was no striking difference in the skin reactions produced by the strains used. Only one of the animals died, but at autopsy no lesions specific for toxoplasmosis could be found, so that an intercurrent death is suspected. Evidence of cross-immunity has been given by this experiment.

As no differences in behavior, morphology, virulence, cross-neutralization and cross-immunity between the strains Bk and S could be observed, we believe that they are closely related or even may be identical.

The infant died on September 21. Treatment with sulphathiazole was not successful. At autopsy Dr. H. E. Schornagel found toxoplasma-containing mononuclear infiltrations in the meninges, the walls of the ventricles and the cerebral tissue, and foci of necrosis with many mononuclear cells and extracellular parasites. In the subcutaneous tissue, the psoas muscle and the diaphragma, some toxoplasma-containing mononuclear infiltrations were found.

Summary. This is a report of a case of toxoplasmosis occurring in Holland in a six-week-old infant, in whom during life toxoplasma could be shown in the cerebrospinal fluid by smear and also isolated by mouse inoculation. The serum of the infant's mother showed a strongly positive neutralization reaction, which was carried out by Sabin's rabbit skin test. In cross-neutralization tests and cross-immunity experiments on rabbits, the Dutch strain appeared to be closely related, perhaps identical with an American one.

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Diffusion Constants of the *E. coli* Bacteriophages.

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For determining the diffusion constants of substances which can be obtained pure in relatively large amounts, the conventional optical method of Lamm is most suitable. Unstable substances like bacteriophages and animal viruses, however, are often irreversibly altered by the purification procedures, and diffusion measurements on such systems are then subject to large errors. The diffusion of such substances is better investigated using impure solutions that are quantitatively evaluated through measurements of their biological activities.

Hershey, Kimura and Bronfenbrenner[1] have estimated the relative sizes of T^1 and T^2

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[1] Hershey, A. D., Kimura, Frances, and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 7.