TABLE V.
Effect of Pyruvate and Fumarate upon Antibacterial Activity of Streptomycin.
Incubation 17 hours at 28°C.

Carbon source	*E. coli*		*A. aerogenes*		*P. vulgaris*		*S. aureus*	
	0*	+	0	+	0	+	0	+
None	15	0	18	0	4	0	24	0
Pyruvic acid	36	29	40	4	36	26	44	13
Fumaric acid	40	23	46	6	47	42	22	6
Glucose	37	0	56	0	14	0	44	0

* 0 = no streptomycin; + = 4 μg/ml streptomycin.

acids may counteract the antibiotic effect by acting as essential metabolites in the synthesis of these compounds has been suggested.[5] Certain preliminary results obtained in oxidation experiments, by the use of the Thunberg and Warburg techniques, indicated that streptomycin does not affect metabolic reactions measured in terms of oxygen uptake or dehydrogenase activity; rather it appeared that streptomycin affects a synthetic process, and the effect on respiration is only a secondary phenomenon.

Summary. The addition of 1% pyruvate and fumarate to nutrient broth supported the growth of *E. coli* in the presence of 10 μg/ml of streptomycin. When the concentration of the acids was increased to 3%, growth took place even in the presence of 150 μg/ml of streptomycin. The streptomycin was not destroyed. The test bacteria growing in the presence of streptomycin and of the organic acids did not become resistant to streptomycin.

Salts of succinic, formic, malic, and maleic acids also exerted some antagonistic effect upon streptomycin. Lactose, as well as lactic, acetic, and propionic acids, glycerol, glycerophosphate, and glucose had no effect upon the growth-inhibiting action of streptomycin on *E. coli.*

Comparison of the antagonistic effect of pyruvic and fumaric acids against the streptomycin action upon various bacteria, showed considerable variation. *E. coli* and *P. vulgaris* were largely protected by the organic acids against the action of streptomycin, whereas *A. aerogenes* and *S. aureus* were only slightly affected.

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Urinary Excretion of 17-Ketosteroids by Normal Young Men During Starvation.*

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There are only a few reports indicating the effect of starvation on the urinary excretion of 17-ketosteroids. In most of these, owing

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to a number of complicating factors, it is impossible to assess the influence of the restricted food intake on the urinary excretion.

Dingemanse and her associates[1] found a marked decrease in the concentration of 17-ketosteroids in the urine of the inhabitants of the Netherlands during the German occupation. As they point out, although the content per unit volume was lower during the war than afterwards, the absolute amounts excreted in the two periods may not have been very different since the urine volume during the occupation was 1.5 to 2 times normal.

Salter *et al.*[2] noted a slight decrease in the mean urinary excretion of 17-ketosteroids among 48 repatriated American prisoners-of-war from the Pacific theater. All of these men had gynecomastia at one time or another. The biochemical manifestations of gynecomastia in these ex-P.O.W.'s were said to have been very similar to those observed in similar cases among civilians where the breast hypertrophy and tumor was presumably due to causes other than malnutrition. The papers by the Yale workers give the impression that the decreased 17-ketosteroid excretion is not a manifestation of starvation *per se*.

Chou and Wang[3] reported that the excretion of male sex hormones (as determined by the same general technique used in the 17-ketosteroid analysis) was markedly reduced in malnourished patients in proportion to the degree of undernutrition. However, factors other than starvation must have been operative in these cases since their well-nourished patients showed a rate of excretion of male sex hormones only 42% of the mean for their normal subjects.

Methods. The amount of total neutral 17-ketosteroids in the urine was determined by the micro-method of Miller and Mickelsen.[4] This involved the simultaneous hydrolysis of the urine with hydrochloric acid and extraction with carbon tetrachloride. After a series of washings with potassium hydroxide, hydrochloric acid and water, the carbon tetrachloride extract was evaporated to dryness *in vacuo*. The residue was dissolved in ethyl alcohol and then subjected to the usual Zimmermann reaction. In all cases duplicates were carried through from the beginning of the procedure and repeated when they failed to agree within 4%. All urine specimens were collected in bottles containing glacial acetic acid as a preservative. The analyses were started within 24 hours of the time of collection, or the sample was stored at −20°C until the analysis could be made.

Semi-starvation experiment. Thirty-four normal young men between 19 and 32 years of age voluntarily submitted to a semi-starvation experiment. During the 6-month period when they were maintained on an average daily intake of 1600 calories, they lost 24% of their original body weight. The diet simulated that available to the inhabitants of north-central Europe and was relatively adequate in all determinable factors other than calories and protein. Before the experiment started, the subjects were put through various physical and psychological tests in order to exclude those who deviated from normal. Other details of this experiment have been described elsewhere.[5]

A random group of 10 of these men was chosen for the study of the 17-ketosteroid excretion. The semi-starvation phase of the experiment was preceded by a 3-months control period. No urine samples were collected for these special analyses at that time. However, control samples were collected from some of the men on the 57th week of rehabilitation when the subjects were back to normal in all respects. Additional control urinary excretion values from a large group of similar young men were available for comparative purposes.

Urine samples were collected for the deter-

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[3] Chou, C. Y., and Wang, C. W., *Chinese J. Physiol.*, 1939, **14**, 151.

[4] Miller, E. v. O., and Mickelsen, O., to be published.

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TABLE I.
Urinary Excretion of 17-Ketosteroids During Semi-starvation and Subsequent Rehabilitation. All values are expressed as mg of androsterone per 24 hours. The mean urine volumes as cc per 24 hrs are given. The standard deviations are given with the means.

Subject	S8	S24	R6	R57
1	10.8	10.2	17.6	12.6
9	10.8	9.8	12.8	15.0
12	3.3	8.7	10.9	11.7
104	7.7	6.9	9.8	11.2
122	7.0	6.4	9.6	10.3
Mean	7.92 ± 3.12	8.40 ± 1.70	12.14 ± 3.31	12.16 ± 1.79
23		4.7	10.7	13.0
26		7.7	18.2	10.1
109		8.0		11.0
130		11.5	5.8	17.5
232	8.6	8.8		
Mean (all subjects)	8.03 ± 2.80	8.27 ± 1.99	11.92 ± 4.18	12.49 ± 2.42
Urine vol. (all subjects)	1848 ± 759	3009 ± 1794	3838 ± 925	1380 ± 383

mination of the ketosteroid excretion at the following times: (1) the eighth week of starvation (S8) when the men had lost an average of 13% of their original body weight, (2) at the twenty-fourth week of semi-starvation (S24) when they had lost 24% of their original body weight, (3) at the sixth week of rehabilitation (R6) when the men had regained some of their lost weight and (4) at the 57th week of rehabilitation (R57) when they were back to normal in all respects.

Acute starvation experiment. Twelve normal young men were subjected to a 4½-day period when they received no food. Water was the only substance permitted by mouth during this period and it was unrestricted. Before and after this, these men were the subjects of a long-time experiment during which they received diets controlled as far as calories and thiamine were concerned. Throughout the starvation period, the physical activity of the subjects was maintained by standardized walking on a motor-driven treadmill. The work was of such intensity that a calculated caloric deficit of approximately 5000 calories per day was produced. The training factor involved in this high plane of activity was cancelled out since these men had done the same amount of work for a week or more preceding the fast.

Urine samples for the estimation of the 17-ketosteroid excretion were collected on the third and fourth days of the fast. The control samples were secured some months later. These values agreed with those secured a number of months preceding the experiment. The validity of these control samples has been independently proven by the constancy of the 24-hour urinary excretion of total, neutral 17-ketosteroids over periods as long as a year or more.[6,7]

Results—semi-starvation experiment. The excretion of total, neutral 17-ketosteroids for the 5 men studied at S8 averaged 7.9 mg per 24 hours (Table I). This is considerably below the mean excretion level of 11.3 mg observed for normal young men in this laboratory[7] as well as the 12.2 mg for these men at the 57th week of rehabilitation. Each subject at S8 showed a lower excretion than that during the recovery period. In fact subject 12 showed an excretion level considerably below any observed by us in normal young men, while the values for the rest of the subjects were in the lower normal range.

At S24, the 17-ketosteroid excretion of the above group showed no essential change except for subject 12 whose value increased considerably. The values for the other men were essentially the same as those at S8. Five additional men studied at S24 showed the same decrease in 17-ketosteroid excretion as the preceding group.

During the rehabilitation period when the

[6] Miller, E. v. O., Mickelsen, O., and Keys, A., to be published.

[7] Miller, E. v. O., Mickelsen, O., and Keys, A., *Fed. Proc.*, 1947, **6**, 279.

TABLE II.

The Urinary Excretion of 17-Ketosteroids During Acute Starvation. All values for ketosteroid excretion are expressed as mg of androsterone per 24 hours. The mean urine volumes as cc per 24 hours are given. The standard deviations are given with the means. The decrease in ketosteroid excretion observed on the fourth day of starvation is expressed as a percentage of the control value (% Max. Decr.)

Subject	Control period	3rd day starvn.	4th day starvn.	% Max. Decr.
B	11.7	5.2	4.7	59.8
A	12.4	5.7	2.9	76.6
M	11.3	4.1	3.4	69.9
D	9.1	4.3	3.0	67.0
Pa	9.9	3.6	2.7	72.7
Pe	13.8	4.9	3.7	73.2
C	10.9	3.8	3.2	70.6
H	12.9	4.7	4.7	63.6
E	12.8	5.2	4.3	66.4
W	9.3	2.7	2.3	75.3
S	9.6	3.3	2.6	72.9
R	13.3	4.6	5.5	58.6
Mean	11.41 ± 1.66	4.34 ± 0.88	3.58 ± 1.00	68.9
Urine vols.	902 ± 245	745 ± 258	813 ± 445	

food intake was raised, the ketosteroid excretion increased. By R6, the mean excretion level for the 8 men examined then was 11.9 mg. The ketosteroid excretion returned practically to normal in spite of the fact that in even those subjects receiving the largest amount of food, body weight restoration amounted to a maximum of 18% of that lost during starvation with the values for most of the men below 7%. The recovery of physical fitness at this time was also very low. A subsequent year on an unlimited diet produced only a negligible increase in the 17-ketosteroid excretion. Subject 130 whose excretion was below normal at R6 was the one whose food intake during this period was the lowest. His recovery, on the basis of such factors as restoration of body weight, improvement of physical performance, etc., was considerably less than that of the other subjects. This subject was unusual in that his excretion level at R6 was so very much lower than the value at S24. In 2 cases (1 and 26) the excretion at R6 was much greater than at R57. There is no apparent explanation for these findings.

Results—acute starvation experiment. The 3 days of acute starvation produced a marked reduction in the 17-ketosteroid excretion of all 12 subjects (Table II). The average excretion during the control period was 11.41± 1.66 mg per 24 hours whereas that for the third day of the experiment was 4.34±0.88 and that for the fourth day was 3.58±1.00. In practically every case, the 24-hour urinary excretion was below the lower normal limits. On the fourth day of starvation, the ketosteroid output was still lower than that of the preceding day in all but 2 cases.

By far the greatest part of the reduction in the urinary excretion occurred by the third day of starvation. The decrease by that time averaged 62% of the control values whereas the total reduction was 69%. The reduction of the ketosteroid excretion ranged from 59 to 77%. There was no relation between the magnitude of the reduction and the control ketosteroid level.

The rate of excretion of ketosteroids in these two types of starvation was not influenced by the urine volume. In the semi-starvation experiment during the entire starvation phase the daily urine volume was about twice the control value. In the acute starvation experiment, on the other hand, the urine volume was markedly reduced during the starvation period.

Discussion. Both acute and semi-starvation with hard work produced marked reductions in the rate of total, neutral 17-ketosteroid excretion, the influence of acute starvation being much greater than that of semi-starvation. Any attempt to explain this change must involve a consideration of the functions influenced by each of the experiments. The plane of metabolism cannot be

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