the anti-B agglutinins of group A and O individuals. The pooled sera† in almost every case were found to have titers and avidities approximating those of the strongest sera in the group. However, this point cannot be stressed much since the pooled sera were assayed against a different batch of red cells.

Conclusions. The increase in agglutination titer following immunization confirms the work of Witebsky[1] and Wiener.[2] In addition it has been shown that immunization with group specific substances increases the avidity, and that this increase roughly parallels the increase in agglutinin titer.

† We are very grateful to Dr. Wm. C. Boyd of Harvard University for comparing our pooled sera with the Harvard reference standards[5] obtained as byproducts of plasma fractionation. The titers and avidities obtained by Dr. Boyd compare favorably with the reference standards. We also wish to thank Dr. Ernest Witebsky for kindly confirming the results of our assays.

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Relationship of Turbidity to Acid Production by *Lactobacillus arabinosus.*

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In microbiological assays using the *Lactobacillus arabinosus* either turbidity or total acid production may be used as a measure of the response to the organism to various growth factors. Since, in all tests for which the organism is used, the turbidity resulting from bacterial growth increases with the amount of acid produced, it has generally been assumed that the turbidity corresponding to a given amount of acid production would be the same regardless of the growth factor to which the organisms were responding. In the course of some determinations of nicotinic acid, pantothenic acid, and biotin it was noted that the turbidity was much greater in relation to acid production when the organisms were responding to suboptimal amounts of nicotinic acid than when responding to suboptimal amount of biotin or calcium pantothenate.

Methods. All tests were carried out in triplicate except those involving *l*-tryptophane† which were done in duplicate. The organisms were grown in Evelyn photometer tubes in 10 cc of the medium of Snell and Wright[1] as modified by Krehl, Strong, and Elvehjem,[2] omitting nicotinic acid, biotin, pantothenate, or *l*-tryptophane as the case might be. Stock cultures were carried and organisms were prepared for use as inocula as described by Snell and Wright.[1] Turbidity was measured in the Evelyn photoelectric colorimeter using filter 660. The total acid produced was titrated with 0.1 N sodium hydroxide. Lactic acid was determined by the method of Barker and Summerson.[3] These determinations were carried out in the biotin, nicotinic acid, and pantothenate tests after 18, 42, and 66 hours incubation at 37°C. In the tryptophane tests only the 66-hour determination was made and lactic acid production was not measured. The response to nicotinic acid was studied over the range from 0.1 to 1.0 μg, biotin from 0.1 to 2.0 millimicrograms, calcium pantothenate from 0.02 to 0.5 μg, and *l*-tryptophane from 2 to 10 μg per 10 ml of medium.

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† We are indebted to Dr. J. G. Wooley for these data.

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TABLE I.
Relationship of Turbidity to Acid Production by *Lactobacillus arabinosus*.

Nicotinic acid		Calcium pantothenate		Biotin		*l*-Tryptophane	
μg per 10 ml medium	Ratio* acid to turbidity	μg per 10 ml medium	Ratio* acid to turbidity	Milli-micrograms per 10 ml medium	Ratio* acid to turbidity	μg per 10 ml medium	Ratio* acid to turbidity
0.1	11.95	0.02	81	0.1	51.8	2	45.8
0.2	13.0	0.04	78	0.2	51.6	4	44.4
0.4	14.2	0.1	50.8	0.6	38.3	6	42.4
0.6	16.1	0.3	24.1	0.8	36.3	8	40.0
1.0	16.9	0.5	22.0	2.0	27.2	10	39.4

* Total titratable acidity

2-Log. galvanometer reading

All figures were obtained after 66 hours incubation and are the average of triplicate determinations except in the instance of tryptophane, where they were done in duplicate.

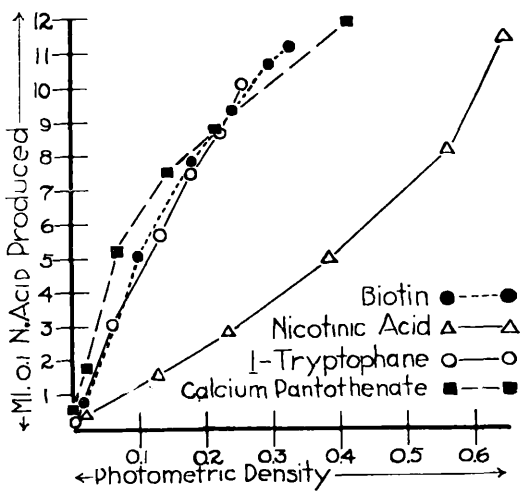
Results. The results obtained at various incubation times were similar and varied only in degree. Therefore, only the results obtained at 66 hours incubation are reported.

The ratios of total acid produced to photometric density were much lower when the organisms were responding to suboptimal amounts of nicotinic acid than when they were responding to suboptimal amounts of biotin, pantothenate, or *l*-tryptophane (Table I). At a level of acid production of 5 to 6 ml of 0.1 N acid (line 2 of Table I) the ratios were 13.0 for nicotinic acid, 78 for calcium pantothenate, 51.6 for biotin, and 44.4 for *l*-tryptophane. This indicates that, at this particular level of acid production, about 6 times as many bacteria were present in the nicotinic acid tubes as in the pantothenate tubes, and 3½ to 4 times as many as in the tryptophane or biotin tubes. As the amount of nicotinic acid was increased the ratios rose, and as the amounts of pantothenate, biotin, and tryptophane were increased, the ratios fell and tended to approach those obtained with nicotinic acid. When photometric densities were plotted against total acid production (Fig. 1), the curves for biotin, pantothenate, and *l*-tryptophane overlapped and were quite similar, whereas the nicotinic acid curve differed from all the others.

Lactic acid accounted for 98 to 102% of the total titratable acid regardless of whether the organisms were responding to nicotinic acid, biotin, or pantothenate.

Discussion. The data show that when biotin, pantothenate, or *l*-tryptophane are present in suboptimal amounts, fewer organisms are formed per unit of glucose metabolized than is the case with nicotinic acid. This suggests that lack of biotin or pantothenate interferes with the synthesis of protein to a greater degree than does lack of nicotinic acid. This view is reinforced by the similarity of the biotin and pantothenate curves to that of *l*-tryptophane. Conversely, a lack of nicotinic acid interferes with carbohydrate metabolism more than does a defi-

Figure 1-
Relationship of Photometric Density to Acid Production by Lactobacillus Arabinosus



ciency of biotin, pantothenate, or *l*-tryptophane.

Though the total fermentation of carbohydrate is reduced when biotin, pantothenate, or nicotinic acid are present in suboptimal amounts, the fact that the end product is always lactic acid suggests that the main pathways of carbohydrate metabolism are unchanged regardless of the growth factor to which the organism is responding.

Summary. 1. The turbidity produced by the growth of *Lactobacillus arabinosus* is greater in relation to acid production when the organisms are responding to suboptimal amounts of nicotinic acid than when they are responding to suboptimal amounts of biotin, pantothenic acid, or *l*-tryptophane.

2. All the acid produced by the *Lactobacillus arabinosus* is lactic acid regardless of whether the bacteria are responding to nicotinic acid, biotin, or pantothenate.

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Changes in Erythrocytes of Hamsters Following Castration, Splenectomy, and Subsequent Liver, Iron, and Testosterone Injections.

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A variety of evidence indicates that the male sex hormone has a stimulating effect upon the red blood cell count in mammals. It is well known that men have a higher average count than women, and a similar sex difference has been found to occur in many other mammals including rats,[1] rabbits,[2] mice,[3] and cats.[4] That this is due, in part at least, to the effect of the male hormone has been demonstrated by the increased count following testosterone injections in hypogonadic men,[5] in capons,[6] and in castrate female and hypophysectomized rats,[7] and by the reduction in number of red blood cells following castration in the hamster (*Cricetus auratus*) reported previously from this laboratory.[8] Some progress has also been made toward an understanding of the mechanism by which the effect of the male hormone is produced. Vollmer and Gordon[7] found increased reticulocytosis and hyperplasia of the bone marrow in hypophysectomized rats injected with testosterone propionate. The present study was undertaken to confirm and extend the results obtained following castration in the hamster[8] and also to attempt to discover further details concerning the processes involved in the effects of castration upon the red blood cells.

Experimental. Nineteen male golden hamsters (*Cricetus auratus*) (Tables I, II) were employed in this experiment, four sets of littermates plus 2 animals from the same strain but of which the exact relationship to each other was unknown. All were of the fourth and fifth generations from an original inbred litter of four animals born in this laboratory in the spring of 1943. They were kept in a temperature of 70 to 75°F and had Purina Dog Chow and/or Pratt's Nurishmix constantly available in their cages, supplemented occasionally by milk and lettuce. They appeared

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