which shows that thyroxin is in all probability not responsible for the immediate growth after adrenalectomy.

Summary. When the hair is removed from the backs of underfed rats it does not grow externally for long periods. Upon adrenalectomy of such underfed rats, the hair grows on their backs in 8 or 9 days. Histological sections show no increased activity of the thyroid in such adrenalectomized animals. When underfed individuals are thyroidectomized one to 21 days previous to adrenalectomy, the hair, likewise, grows in 8 or 9 days after removal of the adrenals. There is, therefore, no evidence that the thyroid is responsible in any way for the rapid growth of hair in the rat following adrenalectomy.

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The Nature of Sulfanilamide Inhibition.*

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The mechanism of the action of the sulfonamides and the nature of the inhibition of their bactericidal effect by a number of compounds has not been clearly demonstrated. The discovery of the anti-sulfanilamide effect of p-amino benzoic acid by Woods[1] has opened a new approach to the problem. Fildes[2] explained this action as the inactivation by the sulfonamide of an essential enzymic grouping involving in some way p-amino benzoic acid. Support for this thesis was advanced when Rubbo and Gillespie[3] showed the compound to be a growth factor for *Cl. acetobutylicum.* They also showed that large amounts of sulfanilamide (23,000 mols. per mol. of p-amino benzoic acid) would prevent the growth occasioned by the presence of the growth factor. Thus it appears that p-amino benzoic acid may be an essential metabolite, the normal metabolism of which is disturbed by the presence of sulfanilamide.

The competitive nature of the actions of the sulfonamides and p-amino benzoic acid has been discussed by Lockwood.[4] Actual

* The author is indebted to Dr. F. C. Schmelkes for comments and suggestions.

1 Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

2 Fildes, P., *Lancet*, 1940, **1**, 955.

3 Rubbo, S. D., and Gillespie, J. M., *Nature*, 1940, **146**, 838.

4 Lockwood, J. S., *Surg., Gynecol. and Obstet.*, 1941, **72**, 307.

experimental proof of the competition has not been presented. Such proof can be obtained from the following mathematical considerations. If the following equations be assumed:

$$E + S \rightleftarrows E\,S \rightarrow E + P \quad (1)$$
$$E + I \rightleftarrows E\,I \text{ (inactive)} \quad (2)$$

the equations below can be derived.[5]

In the absence of inhibitor

$$\frac{1}{v} = \frac{1}{V}\,[K_s]\;1/(S) + \frac{1}{V} \quad (3)$$

In the presence of inhibitor

$$\frac{1}{v_i} = \frac{1}{V}\left[K_s + \frac{K_s\,(I)}{K_i}\right]\frac{1}{(S)} + \frac{1}{V} \quad (4)$$

where

v = velocity of reaction in absence of inhibitor.
v_i = velocity of reaction in presence of inhibitor.
(S) = concentration of substrate.
(I) = concentration of inhibitor.
(E) = concentration of enzyme.
P = product of reaction (usually the measured quantity).
V = maximum velocity when enzyme is saturated with substrate.
K_s = dissociation constant of enzyme-substrate complex.
K_i = dissociation constant of enzyme-inhibitor complex.

Equations (1) and (2) have been manipulated to produce equations (3) and (4) because these permit the physical constants to be determined from experimental data. It can be seen that if the assumptions are correct (*i. e.*, if inhibition of the competitive type prevails) then, when the reciprocal of the velocity of the reaction is plotted against the reciprocal of the substrate concentration a straight line will result with an intercept of $1/V$ and a slope of $1/V\,[K_s]$ in the absence of an inhibitor and a slope of $1/V\left[K_s + \frac{K\,(I)}{K_i}\right]$ in its presence. Therefore the presence of an inhibitor will not affect the intercept of the line in such a plot but it will increase the slope by the amount $\frac{1}{V} \cdot \frac{K_s\,(I)}{K_i}$.

Experimental. A synthetic medium[6] was inoculated with 2,000,000 *E. coli* per ml and dispensed into test tubes calibrated for light transmission. The substrate p-amino benzoic acid and the inhibitor sulfanilamide were added and the cultures were incubated at 38°C. At intervals the turbidity was measured in a Hellige electrophotometer using a 660 millimicron filter which previous experiments had shown to give turbidity readings directly proportional to the

[5] Elvehjem *et al.*, *Respiratory Enzymes*, Minneapolis, 1939.
[6] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 307.

number of organisms in the range of these experiments. Typical readings from the third to the seventh hour—the period of logarithmic growth—are recorded in Table I. It will be observed that p-amino benzoic acid in itself had no effect upon growth. Sulfanilamide at a level of 10 mg per 100 ml prevented growth entirely over the period of observation. In the presence of 25 mg % of sulfanilamide the addition of .01 mg % p-amino benzoic acid (a molecular ratio of 2,000:1) permits the reaction to proceed at about ½ the maximum rate. This does not take into account p-amino benzoic acid which may have been synthesized by the organism.

The logarithms of the turbidity readings were plotted against time and the slope of the resulting straight lines was used as a measure of the growth rate. The lines were fitted to the experimental points by the "method of the least squares" to obtain the best estimate of the slope (b) and the error (s^2). It will be observed that the errors are very small, indicating an excellent fit of the data to the lines, and that the series of errors is homogeneous. The velocity constant k which is the best estimate of the velocity of the reaction (v), is 2.303 times the slope of the line since common logarithms were used in the plot.

Having determined the velocity of growth in the presence of various concentrations of substrate and inhibitor the reciprocal of

TABLE I.

Effect of Sulfanilamide and Para-amino Benzoic Acid on Growth Rate of E. coli.

Conc. mg%		Turbidity (arbitrary units*)								
Sulfanilamide	P.A.B.	3	4	5	6	7 hrs	b†	k	1/k	s²‡
25	0	.4	.4	.4	.4	.3	—.025	0.00	∞	.0021
25	.01	1.5	2.2	2.6	3.6	5.7	.137	.316	3.16	.0014
25	.03	1.6	2.6	3.8	6.8	11.4	.212	.489	2.04	.0010
25	.05	1.9	3.1	5.5	9.6	16.7	.238	.549	1.82	.0003
25	.1	2.0	3.5	6.1	11.5	20.0	.252	.581	1.72	.0001
25	.3	2.2	4.3	9.0	15.5	27.0	.273	.629	1.59	.0009
10	0	.5	.5	.4	.5	.5	.000	.000	∞	.0025
10	.005	1.7	2.8	4.4	6.9	11.0	.201	.463	2.16	.0001
10	.01	2.0	3.2	6.2	9.6	17.0	.234	.540	1.85	.0006
10	.03	2.3	4.1	7.6	13.4	24.0	.255	.587	1.70	.0000
10	.05	2.3	4.4	8.9	15.0	27.0	.267	.615	1.63	.0006
10	.1	2.3	5.1	10.0	16.9	30.0	.275	.634	1.58	.0018
0	.005	2.5	5.0	10.0	18.0	32.0	.277	.638	1.57	.0006
0	.01	2.3	5.4	10.6	17.6	33.0	.283	.652	1.53	.0023
0	.03	2.4	5.3	10.0	17.8	33.0	.280	.645	1.55	.0010
0	.05	2.4	5.0	10.0	18.1	32.0	.281	.648	1.54	.0008
0	.1	2.4	5.3	10.0	17.8	33.0	.280	.645	1.55	.0010
0	.3	2.4	5.3	10.0	17.6	33.2	.280	.645	1.55	.0010
0	0	2.4	5.2	10.0	18.9	33.0	.283	.652	1.53	.0009

*1 unit = 8 million *E. coli.*

†Slope of plot of log. turbidity vs. time.

‡Error in plot of log. turbidity vs. time.

the velocity was plotted against the reciprocal of the substrate concentration. The results shown in Fig. 1 substantiate the theory of competitive inhibition since (1) the plots give straight lines as postulated, (2) the presence of the inhibitor has no effect on the intercept which remains as the reciprocal of the maximum velocity ($1/V = 1.55 \pm 0.02$) regardless of inhibitor concentration, and (3) the concentration of inhibitor determines the slope of the lines.

The statistical presentation of these data appears in Table II. The intercepts (1/V) of 1.54, 1.57 and 1.53 do not differ significantly. The experimental data fit very well to the straight

TABLE II.
Summary of Statistical Analysis.

Sulfanilamide concentration	N	Equation of line	variance (s^2)	t	$t^*_{0.01}$
mg%					
0	6	$1/k = .0001\ 1/cP.A.B. + 1.54$	.0002		
				17.6	3.50
10	5	$1/k = .0029\ 1/cP.A.B. + 1.57$	.0006		
				12.3	3.70
25	5	$1/k = .0162\ 1/cP.A.B. + 1.53$	.0013		

cP.A.B. = concentration p-amino benzoic acid.
*From Fischer's t table. When t exceeds $t_{0.01}$ the probability is less than 1 in 100 that the difference in slopes is due to chance alone.

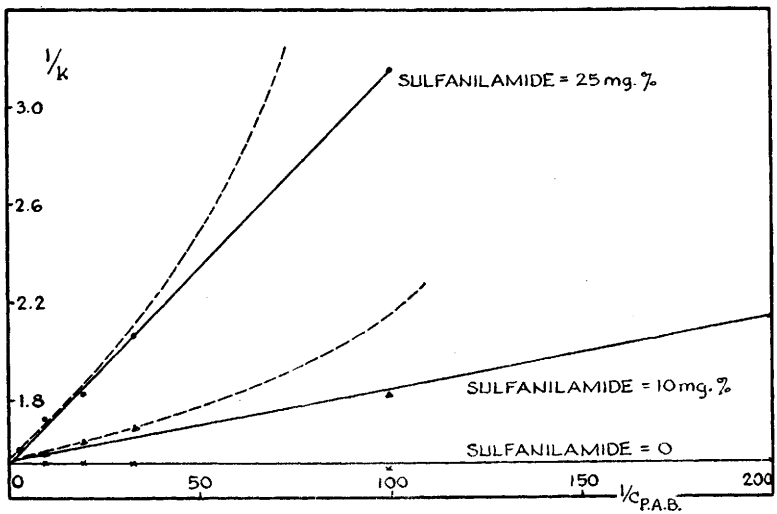

FIG. 1.
Test for Type of Inhibition by Sulfanilamide.
Ordinate = 1/velocity constant. Abscissa = 1/concentration p-amino benzoic acid. Solid lines are fitted to actual experimental points. Dotted lines indicate the plot if the organisms contained 0.005 mg% p-amino benzoic acid in addition to that which was added.

lines as is indicated by the small variance (s^2). Application of the t test[7] reveals that the differences in slopes are far in excess of the requirements for significance.

The fact that maximum growth velocity occurs with *E. coli* in the absence of any added p-amino benzoic acid if sulfanilamide is also absent does not refute the thesis of competitive inhibition. P-amino benzoic acid may be present as an impurity in the medium or more likely, it may be synthesized by the organism as required. That such amounts are very small is demonstrated by the plot of the broken lines in Fig. 1 which show how the graph would appear if 0.005 mg % of p-amino benzoic acid were present, either as impurity or as a product of bacterial synthesis, in addition to the added amount. Obviously such is not the case. However, since Rubbo and Gillespie[8] showed that as little as .00002 micrograms permitted growth of *Cl. acetobutylicum,* the amount required for growth could be present without noticeably distorting the plot. Admittedly, alternative explanations are available. Any one of a number of proposed mechanisms for sulfonamide action may obtain but it is now possible to reject all but those in which competitive inhibition is characteristic. The data presented here clearly demonstrate that sulfanilamide and p-amino benzoic acid behave as if they were competing for the same receptor site in the organism. The tremendous disproportion between the molecular concentrations merely indicates the greater affinity of the site for the p-amino benzoic acid.

Summary. Growth rates of *E. coli* were measured in the presence of sulfanilamide and p-amino benzoic acid. Mathematical analyses of the data indicate that the sulfanilamide inhibition is of the competitive type.

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Pathogenesis of Erythroblastosis Fetalis: Absence of the Rh Factor from Saliva.

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The pathogenesis of erythroblastosis fetalis has been ascribed to the isoimmunization of the mother by the Rh, or, more rarely,

[7] Fisher, R. A., *Design of Experiments,* London, 1937.