factor, redistribution of enzyme from other tissues, and the presence of a simple inhibitor in normal livers or of a simple activator in treated livers. The final decision as to whether any case of increased enzyme activity is due to a corresponding increase in the amount of enzyme should be based upon an estimation of enzyme concentration not depending upon activity. The evidence so far available suggests, however, that this change in activity of the peroxidase-oxidase system represents an increase of the concentration of the components of this system.

Increased activity of the order shown in the accompanying tables can be produced by oral, subcutaneous, or intraperitoneal administration of tryptophan to rabbits, rats, or guinea pigs. Two characteristics of this reaction may be emphasized. The enzyme increases within several hours after administration of an active substance, and after 15–20 hr has returned to normal. Although the blood levels of administered compounds have not been determined, this close correspondence of enzyme levels to those assumed for any compound administered (e.g., histidine [*4*]), bespeaks a remarkably rapid adaptive response and decline. The time course of this change in the enzyme concentration following the intraperitoneal administration of 2 mM of L-histidine to rats is shown in Table 2. Second, this enzyme adaptation is also produced by several substances that are not substrates for the enzyme, notably histidine and kynurenine and to a lesser extent tyrosine and phenylalanine. Although any physiological connection between these substances and the tryptophan oxidizing system in animals is conjectural at present, the specificity of the enzyme response to them is emphasized by the absence of any response to larger amounts of some other substances administered in the same way.

The mechanism involved in the adaptation of this enzyme in animals would appear to be somewhat complex in comparison to enzyme adaptation in simpler forms. The greater organizational complexity of animals may also provide mechanisms for the increase of enzymes in response to compounds other than their substrates, as part of a general physiological adjustment. Several other enzymes have been found which, though not necessarily adaptive, do have activities different from normal in certain physiological states: succinic dehydrogenase (*5, 6*), cytochrome *c* (*7*), proline oxidase (*8*), arginase (*9*), alkaline phosphatase (*10*), and xanthine oxidase (*11*). The more immediate implications of this particular response concern its undoubted effect upon the amount of tryptophan converted to kynurenine and other metabolites *in vivo* (*12*), and its possible role in determining the amount of tryptophan converted to nicotinic acid under various conditions (*13*).

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Experiments on the Catalytic Exchange of Acetone and Propane with Deuterium

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The reduction of acetone to propane with deuterium on platinized platinum was attempted at low temperatures (−20° C) in an effort to prepare $CH_3CD_2CH_3$ as predicted by Farkas and Farkas (*1*). It was hoped that the electron dissociation pattern of this compound would aid in the interpretation of the results of the mass spectrometric examination of the exchange of propane with deuterium.

The experimental method was similar to that used by Farkas (*1*). The catalyst was prepared by platinizing a platinum electrode in the standard manner, using a 3% solution of platinic chloride and .02% lead acetate. It was washed with boiling water, rinsed with acetone, and dried by a stream of nitrogen. In order to prevent contamination by stopcock grease and mercury vapor the catalyst was never kept *in vacuo*. The catalyst was found to remain stable for periods as long as 2 weeks if left in an atmosphere of nitrogen or hydrogen when not in use.

Approximately 7 cm of acetone was admitted to the reactor and frozen with dry ice, after which about 14

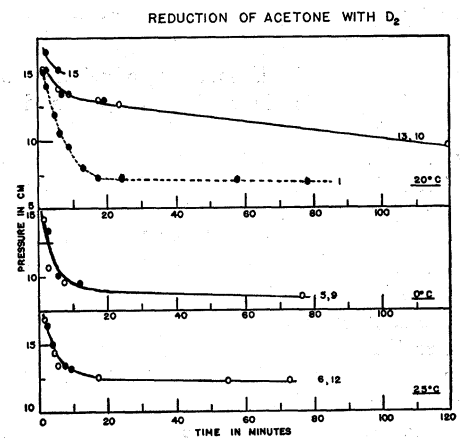

FIG. 1.

TABLE 1

REDUCTION OF ACETONE WITH D_2 PARTIAL MASS SPECTRA OF PROPANES FORMED

Temperature			−20° C			0° C		25° C	
Run	15	13	10	1	9	5	12	6	C_3H_8
Time (min)	6	20	120	75	9	74	9	73	
% Reduction	30	40	50	70	55	65	60	60	
m/e									
29	120	150	160	—	240	350	270	340	430
30	260	270	280	—	390	490	420	450	8.4
31	360	360	340	310	440	430	410	420	—
32	55	51	63	60	130	190	170	230	—
33	10.5	8.6	9.7	15	37	85	67	82	—
34	—	4.9	4.5	4.1	16	42	32	41	—
44	48	48	45	(95)	72	83	78	83	*100*
45	36	39	42	(59)	62	92	74	85	1.9
46	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	—
47	28	25	26	30	49	57	63	67	—
48	6.6	5.9	6.3	6.0	18	35	36	37	—
49	3.3	2.4	2.9	2.3	8.4	22	18	22	—
50	1.7	1.3	1.6	1.3	4.5	15	14	16	—
51	0.9	0.6	0.7	0.4	2.1	7.6	6.6	7.7	—
52	0.6	0.4	0.6	0.1	1.3	4.4	3.2	4.1	—

cm of deuterium (99% D_2) was admitted. An ice-salt bath at −20° C was then placed around the reactor for the duration of the experiment. The drop in pressure of the system is plotted against time at this and other temperatures in Fig. 1. At the completion of the run the reaction was stopped by freezing the mixture with liquid air. The amount of unreacted hydrogens was given by the residual pressure. After this was removed, the pressure of propane was obtained using a dry-ice trap. Samples of this propane were taken for mass spectrometric examination. All the results given in Fig. 1 and Table 1 were obtained with the same catalyst. The catalytic activity of the platinum decreased gradually with use following Run 1, in which it was considerably more active.

Mass 46 taken as 100 in Table 1 is the parent peak of $CH_3CD_2CH_3$. Masses up to 52 were obtained, indicating that all the deuterated propanes up to C_3D_8 were formed. This is different from the results reported by Farkas and Farkas (*1*). Even at −22° C exchange with acetone took place. This exchange was more rapid than the reduction, since the mass spectra indicate that the distribution of deuterium in the propane is the same at both 30% and 70% reductions. At 0° C, exchange can be seen to increase with time before reduction reaches completion. At 25° C the exchange and reduction of acetone are almost complete in 9 min. The slight increase in the deuterium content of the propane can be attributed to its exchange with deuterium.

Since the platinized platinum used might have contained traces of lead, which could promote the exchange reaction, precipitated platinum black was prepared. This catalyst gave essentially the same results.

Recently Turkevich and Friedman (*3*) have reported their studies with platinum charcoal catalysts, in which similar results were found.

Our experiments were discontinued when it was found that the reaction did not lead to the formation of pure $CH_3CD_2CH_3$, and therefore would not aid in the study of the exchange reactions with propane.

The exchange of propane with deuterium in the presence of catalysts has been studied previously, using thermal conductivity and infrared methods of analysis. Morikawa, Benedict, and Taylor (*4*) worked with the series methane, ethane, and propane on active nickel surfaces and found exchange at temperatures above 180°, 110°, and 65° C, respectively, and at pressures of about ½ atmosphere. A. Farkas (*5*) showed in further studies that propane and butane undergo exchange with deuterium on platinized platinum foil at pressures of 40–50 mm in the temperature range 26°–126° C. All hydrogen atoms were assumed to take an equal part in the exchange. In our present work, it was hoped that by use of the mass spectrometer any differences in the exchange rate at the primary and secondary carbon atoms could be noted.

The reactions were carried out under high vacuum conditions in a 200-cc reaction vessel in which platinized platinum gauze (about 12 cm × 4 cm) was suspended. The temperature was controlled to within ± 0.3° C. In each case approximately 14 cm of propane and 14 cm of deuterium were used. Samples to be analyzed were withdrawn after discarding a volume equal to the dead space. The propane was first frozen with liquid nitrogen to permit the rate of the exchange to be followed by thermal conductivity measurements of $H_2 - D_2$. The microthermal conductivity cell used was a modification of Melville and Bolland's (*6*). The deuterated propanes were then studied in a Nier-type mass spectrometer.

Results are summarized in Table 2 and Fig. 2. The kinetics of the reaction agree reasonably well with the results of Farkas (*5*)—i.e., first order with respect to deuterium. However, the energy of activation for our catalyst was 25 kcal as compared to about 12 kcal obtained by Farkas. The rate constants range from 0.001 min^{-1} at 19.8° C to 0.017 at 40.4° C.

TABLE 2

PARTIAL MASS SPECTRA OF THE DEUTERATED PROPANES

m/e	C_3H_8	Run F*			Run G*			Run H*		
		18 Min	30 Min	62 Min	7 Min	59 Min	121 Min	6 Min	40 Min	90 Min
29	430	460	440	430	440	460	470	440	440	480
30	8.4	40	41	70	34	79	130	43	130	260
31	—	12	14	33	8.0	42	84	18	86	174
32	—	6.5	7.6	19	5.7	27	54	12	52	95
33	—	2.5	2.9	6.0	1.2	11	26	5.3	22	36
34	—	0.8	1.1	0.5	0.1	4.2	8.1	2.3	7.8	12
44	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*	*100*
45	1.9	9.6	11	17	8.6	18	26	15	27	49
46	—	2.3	2.9	7.1	1.2	7.9	15	3.1	17	35
47	—	0.8	1.3	4.5	0.4	5.3	11	1.9	12	24
48	—	0.2	0.6	2.3	0.1	3.5	6.7	1.1	7.5	15
49	—	—	0.1	1.0	—	1.5	3.5	0.4	4.3	8.3
50	—	—	—	0.4	—	0.7	1.7	0.1	2.2	3.5
51	—	—	—	—	—	0.1	0.4	—	0.5	1.1
52	—	—	—	—	—	—	—	—	—	0.1

* Runs F, G, and H were made consecutively on the same catalyst preparation.

As can be seen from Table 2, exchange with all hydrogens of propane took place as predicted by Farkas (*5*) and by Taylor (*4*). The great difficulty in the interpretation of this data is that, since there is so much exchange, masses which are the deuterated peaks such as 45, 46, 30, or 31 are not due to one compound but several isomers and fragments of all the deuterated propanes. To detect orientation effects, therefore, corrections must be made.

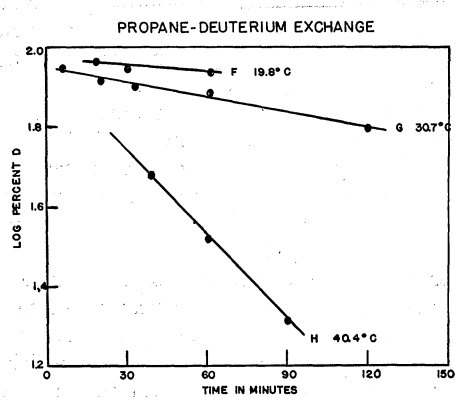

FIG. 2.

If exchange led to a monodeuterated propane, the detection of the position of exchange would be unambiguous. Mass 44 is the parent ion peak ($CH_3CH_2CH_3^+$) of propane. One of the most probable events on electron impact is the loss of a methyl group, leaving mass 29, $CH_3CH_2^+$. From the mass spectrum of propane the ratio $\frac{\text{Mass 29}}{\text{Mass 44}} = 430/100$. If exchange of deuterium with hydrogen in the propane led to CH_3CHDCH_3, the parent peak would be mass 45, and loss of a methyl group would lead to CH_3CHD^+, mass 30. The ratio of $\frac{\text{Mass 30}}{\text{Mass 45}}$ by analogy would be about 4.30. On the other hand, if exchange lead to $CH_2DCH_2CH_3$, the parent peak would still be 45, but electron impact would, with equal probability, lead to $CH_2DCH_2^+$, mass 30, or $CH_2CH_3^+$, mass 29; therefore the ratio $\frac{\text{Mass 30}}{\text{Mass 45}}$ would be expected to be about 2.2. In Fig. 3, mass 30 is plotted against mass 45 for the 2 monodeuterated propanes.

As mentioned above, corrections must be made on the samples obtained from propane-D_2 exchange in order to determine the position of the deuterium atoms. Mass 45, for instance, can be attributed not only to $C_3H_7D^+$ but also to $C_3H_5D_2^+$; $C_3H_3D_3^+$; $C_3HD_4^+$; $C_2C^{13}H_8^+$. Mass 30 is similarly composed of a number of ion fragments. In order to use the relationship indicated above and in Fig. 3, all contributions of the polydeuterated compounds must be eliminated. This has been qualitatively attempted by assuming that the mass spectra of the deuterated propanes are the same as that of normal propane with appropriate mass changes, based on statistics, and the added assumption that the rupture of a C – D bond is one half as likely as the rupture of a C – H bond (*7, 8*).

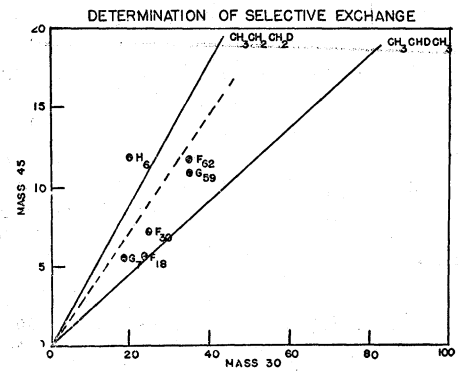

FIG. 3.

This analysis was carried out for several samples, and the results are plotted in Fig. 3. The points fall mainly between the theoretical curves for the 2 monodeuterated propanes, CH_3CHDCH_3 and $CH_3CH_2CH_2D$. A statistical distribution for the

hydrogen-deuterium exchange is 3:1 (primary:secondary) which is the dotted line in Fig. 3. The results show that the distribution is more nearly 1:1. This indicates that deuterium exchanges preferentially at the secondary position. A quantitative measure of this effect must await further detailed information about the electron dissociation patterns of the individual deuterated compounds.

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Adrenal Cholesterol in the Scorbutic Guinea Pig

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Many different stress situations have been shown to evoke in the adrenal of normal animals a response similar to that which occurs following injection of adrenocorticotrophic hormone (ACTH). This response is characterized by a marked fall in the adrenal concentrations of both ascorbic acid and cholesterol. Among the stimuli that have been studied in this regard are the following: hemorrhage (*1*), exposure to cold (*2*), electrical stimulation of peripheral nerves (*2*), burns (*3*), and infection (*4*). Considerations that have been elaborated elsewhere (*5, 6*) appear to justify the use of this response as an indication of the secretion of cortical hormones.

In the present work a study has been made of the effect of stress caused by vitamin C deficiency on the level of cholesterol in the adrenal. Paired-feeding was used in order to distinguish the effect of the vitamin deficiency per se from that of the low calorie intake which accompanies the deficiency in its advanced stages. Two stages of the vitamin deficiency have been studied, both of which are characterized by severe adrenal depletion of vitamin C. The first stage is designated as "early scurvy" and the second as "late scurvy." In the former, inanition has not become a complicating factor in the disease, whereas in the latter, very low food intake accompanies the deficiency. The adrenal is approximately 95% depleted of its vitamin C in the first stage and 98% in the second.

Early scurvy is herein defined as that stage of scurvy occurring when the growth curve reaches a plateau, there is beginning failure of appetite, and the concentration of ascorbic acid in the adrenal is less than 10 mg %. This condition was reached in the present experiments by 17 days after the last vitamin C injection. The concentrations of ascorbic acid in various tissues of early scurvy animals compared with those of late scurvy and normal guinea pigs are shown in Table 1.

TABLE 1

TISSUE VITAMIN C LEVELS IN NORMAL, EARLY SCURVY, AND LATE SCURVY GUINEA PIGS
(Mg/100 g)

	Normal guinea pigs		Early scurvy† pigs		Late scurvy‡ pigs
	Before ACTH	After ACTH§	Before ACTH	After ACTH§	
Adrenal	164.7 ± 7.0*	90.4 ± 4.9	6.02 ± 2.00	6.33 ± 0.85	1.87 ± 0.53
Brain	20.4 ± 0.8	18.0 ± 1.0	8.93 ± 1.10	8.10 ± 0.52	3.21 ± 0.10
Testis	36.5 ± 1.1	34.7 ± 1.9	6.76 ± 0.62	6.42 ± 0.31	2.90 ± 0.32
Spleen	53.8 ± 1.9	44.6 ± 1.7	3.84 ± 0.66	3.69 ± 0.25	.66 ± .12
Kidney	9.19 ± 0.38	7.81 ± 0.79	.59 ± .07	.66 ± .04	.29 ± .06
Liver	19.2 ± 1.1	20.2 ± 2.2	.51 ± .11	.72 ± .15	.25 ± .05
Whole blood	0.62 ± 0.07	0.59 ± 0.05	.07 ± 0.01	.09 ± 0.01	.04 ± 0.02

* Standard error of mean.
† Sacrificed 17 days after last vitamin C injection.
‡ Sacrificed 27–37 days after last vitamin C injection.
§ Sacrificed 6 hr after intraperitoneal injection of 1 mg/100 g of body weight.

Late scurvy is defined for the purposes of this experiment as that stage of scurvy occurring when the vitamin C of such tissues as brain and testis, which are the most slowly depleted of any tissues analyzed in this work, has reached a level below which further significant reduction is difficult to demonstrate; i.e., 80% depletion for brain tissue and 90% for testis. This stage was reached 27 days after the last vitamin C injection. At this time there has been rather rapid weight loss for 9 or 10 days. Gross signs of scurvy are apparent.

Guinea pigs varying in weight from 350 to 500 g, caged individually, were kept at an environmental temperature of 82° ± 2° F and fed a diet (*7*) containing calf meal, 91%; wheat germ, 3%; dried brewers yeast, 3%; and cod liver oil, 3%. Vitamin C was supplied by daily subcutaneous injections of neutralized ascorbic acid (Cevalin #319, Lilly) equivalent to 5 mg/100 g of body weight. Only animals which grew well and remained free of respiratory infections were used.

In order to ensure a state of "tissue saturation," each animal was given a preliminary series of daily injections for at least one week prior to beginning the deficient regime. Each animal on the deficient regime

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