values approximated to those observed in non-proliferating suspensions of the organisms in the presence of the lactic acid or glucose present during growth.

3. Pantothenate inactivation by non-prolifera-

ting bacteria is thus not induced by an imbalance in metabolism resulting from the presence of a growth factor without opportunities for growth. Pantothenate synthesis may proceed from β -alanine or pantoic acid.

REFERENCES

- Benesch, R. (1945). *Lancet*, **1**, 718.
 Dagley, S. & Hinshelwood, C. N. (1938). *J. chem. Soc.* p.1930.
 Emerson, G. A. & Wurtz, E. (1944). *Proc. Soc. exp. Biol., N.Y.*, **57**, 47.
 Fildes, P. (1940). *Brit. J. exp. Path.* **21**, 67.
 Hutchings, B. L., Stokstad, E. L. R., Bohonos, N. & Slobodkin, N. H. (1944). *Science*, **99**, 371.
 Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
 Lwoff, A. & Lwoff, M. (1937). *Proc. Roy. Soc. B*, **122**, 360.
 McIlwain, H. (1944). *Biochem. J.* **38**, 97.
 McIlwain, H. (1946). *Biochem. J.* **40**, 67.
 McIlwain, H. & Hughes, D. E. (1944). *Biochem. J.* **38**, 187.
 McIlwain, H. & Hughes, D. E. (1945). *Biochem. J.* **39**, 133.
 Morel, M. (1941). *Ann. Inst. Pasteur*, **67**, 285, 449.
 Sevag, M. G. & Green, M. N. (1944). *J. biol. Chem.* **154**, 719.
 Thompson, R. C. (1942). *Univ. Texas Publ.* no. 4237, p. 87.
 Wright, L. D. & Welch, A. D. (1943). *Science*, **97**, 426.

Pyruvate Oxidation in Pigeon Brain Catalyzed by Fumarate

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Small amounts of C_4 -dicarboxylic acids, when added to finely minced or ground animal tissues, respiring *in vitro*, are well known to stimulate the O_2 uptake; the effect was first noted by Szent-Györgyi and his co-workers, using pigeon breast muscle (Gözszy & Szent-Györgyi, 1934; Annau, Banga, Gözszy, Huszak, Laki, Straub & Szent-Györgyi, 1935). It was found to be a characteristic of these acids that, unlike true substrates, they preserved the respiration rate of the tissue instead of raising it above its initial level. Stare & Baumann (1937) showed that the effect was truly catalytic; using pigeon breast muscle, they found that the increased O_2 consumption was greater than could be accounted for by the complete oxidation of the added C_4 -dicarboxylic acid. Annau, Banga, Blazso, Brukner, Laki, Straub & Szent-Györgyi (1936) and Annau & Erdös (1939), using rabbit liver and pig kidney, respectively, have demonstrated that catalysis by succinate and fumarate is markedly greater when pyruvate is being oxidized. Other investigations (Banga, Ochoa & Peters, 1939; Krebs & Eggleston, 1940; Smyth, 1940) have shown fumarate catalysis to be active in other tissues and have confirmed its relation to the pyruvate oxidation system.

Although the stimulation by fumarate of the O_2 uptake of tissues respiring in a medium containing pyruvate is now well recognized, it is surprising to find lack of agreement as to whether the rate of pyruvate utilization is also increased. Unfortunately, most determinations of pyruvate disappearance

have been made in the presence of malonate and the literature contains few observations in the absence of this inhibitor. In those cases where malonate has been excluded and the rate of pyruvate disappearance estimated in the presence and absence of fumarate, the observations are conflicting. Thus in pigeon brain, Banga *et al.* (1939), in two experiments quoted, found an increased pyruvate utilization in the presence of fumarate. On the other hand, Annau *et al.* (1936), using rabbit liver, consistently found a decreased rate of disappearance of pyruvate when fumarate was present, while Krebs & Eggleston (1940), working with pigeon breast muscle, reported an increased disappearance in one case and a decreased disappearance in another.

The present investigation is a continuation of previous work (Long, 1943) in which the behaviour of a dialyzed preparation of ground pigeon brain, respiring in a medium containing pyruvate, inorganic phosphate and adenine nucleotide, was quantitatively investigated. With this enzyme system, which is essentially the same as that of Banga *et al.* (1939), the effect of fumarate on the oxidation of pyruvate has now been re-examined. The increased O_2 uptake due to fumarate catalysis, first reported by the last mentioned authors, has been amply confirmed, but their further claim to have observed an increased rate of disappearance of pyruvate could not be supported. Indeed, a slightly decreased rate of pyruvate disappearance has always been found,

and it seems reasonable to assume that this is due to the simultaneous oxidation of fumarate to pyruvate, which is known to take place under these conditions (Long, 1945).

In view of this result, much attention has been given to the measurement of the O_2 uptake of the tissue preparation under different conditions; these further experiments have shown that fumarate catalysis can only take place in the presence of inorganic phosphate and adenine nucleotide. The most important conclusion, however, based on a study of the O_2 /pyruvate ratio, is that fumarate catalysis acts by causing *complete* oxidation of that amount of pyruvate which is only *partially* oxidized in its absence.

EXPERIMENTAL

Materials and methods

The specimens of Na pyruvate, Na α -ketobutyrate, Na arsenite, fumaric acid and adenine nucleotide, previously described, were used. The preparation of the dialyzed pigeon brain suspensions, the respiration technique using Barcroft manometers and the estimation of pyruvate are also similar to those described in earlier papers (Long, 1942, 1943, 1945).

RESULTS

(1) *The effect of fumarate on the O_2 uptake and pyruvate disappearance in a phosphate buffered medium. Adenine nucleotide absent.* The absorption of molecular O_2 by dialyzed pigeon brain suspensions, respiring in a medium containing inorganic phosphate and chloride only, has been found to be increased appreciably by addition of either pyruvate (Long, 1943) or fumarate (Long, 1945). In view of this and the known catalytic effect of fumarate on the O_2 uptake of tissues respiring in pyruvate, in certain circumstances, experiments have been carried out to determine whether fumarate catalysis occurs under the conditions described above. The replacement of pyruvate by α -ketobutyrate, which is similarly oxidized by this enzyme system (Long, 1943), has also been studied. The results obtained are shown in Table 1.

It will be observed that the extra O_2 uptake caused by fumarate is less in the presence of pyruvate or α -ketobutyrate than in the absence of substrate. This can be explained as follows. In the absence of substrate, fumarate is oxidized to pyruvate (Long, 1945) which then undergoes further oxidation; the extra O_2 uptake accompanying these two oxidations is quite appreciable (av. 180 μ l./g./hr.). When pyruvate is initially present in the respiration medium, however, its relatively high concentration saturates the pyruvate oxidation system, so that the additional pyruvate formed from fumarate cannot further increase the O_2 uptake. The slightly increased O_2 uptake which is observed (av. 72 μ l./g./hr.) is undoubtedly therefore that amount which accompanies the oxidation of fumarate to pyruvate. Support for this explanation is to be found in the extent of pyruvate formation from fumarate under these conditions, as given by Long (1945). Thus in three published experiments the amounts of pyruvate produced in the presence of 0.008M-arsenite were 4.9, 8.2 and 6.2 μ mol./g./hr. The average value of 6.4 μ mol. pyruvate must have been derived from an equimolecular amount of fumarate. The O_2 uptake required for the oxidation of this amount of fumarate is also 72 μ l./g./hr.

The results obtained using α -ketobutyrate as substrate can be explained by the inhibiting effect of this substance on the further oxidation of pyruvate formed from fumarate, since both α -ketoacids are oxidized by the same enzyme system. This inhibiting effect is well shown by the experiments quoted in Table 2. It will be seen that the extra O_2 uptakes due to pyruvate in the absence of α -ketobutyrate, given by the difference between columns (3) and (1) are 470 and 218 μ l./g./hr. respectively; in the presence of α -ketobutyrate they are only 284 and 51 μ l./g./hr., as given by the difference between columns (4) and (2). Thus α -ketobutyrate inhibits pyruvate oxidation by from 40 to 80%.

Referring back to the data in Table 1, it must be concluded that since fumarate does not increase the net O_2 uptake due to pyruvate oxidation, fumarate catalysis is absent under these conditions.

Table 1. *The effect of fumarate on the O_2 uptake of dialyzed pigeon brain suspensions, respiring in the presence and absence of pyruvate and α -ketobutyrate*

Pigeon brain suspensions dialyzed for 3 hr. against 0.4% KCl at 2°. Pyruvate and α -ketobutyrate, final concentration 0.01M; fumarate, final concentration 0.005M. Respiration conducted at 28°, pH 7.3. Phosphate buffer (0.05M) present.

Substrate	O ₂ uptake (μl./g. fresh tissue/hr.)								
	None			Pyruvate			α-Ketobutyrate		
	Exp.	Control	Fumarate	Diff.	Control	Fumarate	Diff.	Control	Fumarate
1	225	387	162	472	560	88	350	387	37
2	186	384	198	514	570	56	402	427	25
Av.	206	386	180	493	565	72	376	407	31

Table 2. *The effect of α -ketobutyrate on pyruvate oxidation*

Experimental conditions as in Table 1.

Exp.	O ₂ uptake (μ l./g./hr.)			
	(1) No substrate	(2) α -Keto- butyrate	(3) Pyruvate	(4) Pyruvate + α -keto- butyrate
1	500	686	970	970
2	320	509	538	560

The effect of fumarate on the rate of pyruvate disappearance has been determined in two cases (Table 3); less pyruvate is seen to disappear from the respiration medium in the presence of fumarate than in its absence. This result is also explained by the simultaneous formation of pyruvate from fumarate; the presence of excess pyruvate in the medium prevents further oxidation of this amount, since the pyruvate oxidation system is already saturated. This explanation is supported by the fact that the amount of pyruvate formed from fumarate in the presence of 0.008 M-arsenite, which was simultaneously determined in one of these experiments, was found to be almost identical with the difference between the amounts of pyruvate disappearing in the presence and absence of fumarate.

Table 3. *The effect of fumarate on the rate of pyruvate disappearance*

Experimental details as in Table 1.

Exp.	Pyruvic acid (μ mol./g./hr.)			
	Dis- appearing in absence of fumarate	Dis- appearing in presence of fumarate	Diff.	Formed from fumarate in presence of arsenite
1	39.0	26.5	12.5	11.8
2	39.7	23.5	16.2	—

(2) *The effect of fumarate on the O₂ uptake and pyruvate disappearance in the presence of inorganic phosphate and adenine nucleotide.* Banga *et al.* (1939) observed a considerable increase in O₂ consumption

on adding fumarate to a dialyzed pigeon brain suspension, respiring in a medium containing inorganic phosphate, adenine nucleotide and pyruvate; in control experiments, without pyruvate, the effect of fumarate was small. These observations have been confirmed repeatedly in the present work (Table 4) and are to be contrasted with results previously obtained in the absence of adenine nucleotide (Table 1). The occurrence of fumarate catalysis in brain suspensions seems therefore to be dependent on the presence of inorganic phosphate and adenine nucleotide in the respiration medium. It is interesting to note that pyruvate cannot be replaced by α -ketobutyrate in this system, even under conditions which favour activation of α -ketobutyrate oxidation by added adenine nucleotide, i.e. prolonged (8 hr.) dialysis of the enzyme suspension (Long, 1943).

Using this same enzyme system, Banga *et al.* (1939) have also reported that fumarate catalysis is accompanied by an increased utilization of pyruvate; this observation has not been confirmed in the present work. In Table 5 are quoted the results

Table 5. *The effect of fumarate on the extent of pyruvate disappearance in the presence of inorganic phosphate and adenine nucleotide*

Experimental conditions as in Table 4.

Exp.	Pyruvic acid (μ mol./g./hr.)			
	Dis- appearing in absence of fumarate	Dis- appearing in presence of fumarate	Diff.	Formed from fumarate in presence of arsenite
1	30.8	27.0	3.8	2.6
2	36.1	33.2	2.9	4.3
3	42.8	35.4	7.4	10.2
4	37.2	31.3	5.9	8.0
5	42.2	35.6	6.6	8.2
6	52.8	39.0	13.8	10.5
Av.	40.3	33.6	6.7	7.3

obtained in a number of experiments in which the rate of disappearance of pyruvate was determined in the presence and absence of fumarate. In all

Table 4. *The effect of fumarate on the O₂ uptake of pigeon brain suspensions, respiring in pyruvate or α -ketobutyrate; inorganic phosphate and adenine nucleotide present*

Adenine nucleotide, final concentration 0.0003 M. Other experimental conditions as in Table 1.

Substrate	O ₂ uptake (μ l./g./hr.)								
	None			Pyruvate			α -Ketobutyrate		
	Control	Fumarate	Diff.	Control	Fumarate	Diff.	Control	Fumarate	Diff.
1	319	634	315	1157	1988	831	—	—	—
2	356	740	384	1350	2100	750	—	—	—
3	345	708	363	1192	1902	710	—	—	—
Av.	340	694	354	1233	1997	764	—	—	—
4*	115	251	136	—	—	—	271	312	41

* 8 hr. dialysis.

cases the amount of pyruvate disappearing is slightly less in the presence of fumarate than in its absence, and the difference between the two amounts is very nearly equal to the quantity of pyruvate formed from fumarate in the presence of arsenite. Thus it must be concluded that inorganic phosphate-fumarate-adenine nucleotide catalysis, while stimulating the O_2 uptake, is without effect on the rate of pyruvate disappearance.

(3) *The O_2 /pyruvate ratio for phosphate-fumarate-adenine nucleotide catalysis of pyruvate oxidation.* Previous work (Long, 1943) on the respiring enzyme system—dialyzed pigeon brain suspension, pyruvate and inorganic phosphate—has shown that addition of a catalytic amount (0.0003M) of adenine nucleotide (A.N.) increases not only the rates of pyruvate utilization and O_2 uptake, but also the completeness of pyruvate oxidation, as measured by the O_2 /pyruvate ratio and R.Q. Since, as was shown in the previous section, fumarate addition increased the extra O_2 uptake due to pyruvate oxidation only when inorganic phosphate and adenine nucleotide were also present in the respiration medium, it became of considerable interest to determine the completeness of pyruvate oxidation when subjected to the combined catalytic actions of phosphate, fumarate and adenine nucleotide. The estimation of the O_2 /pyruvate ratio under these conditions has given a satisfactory answer to the problem.

Six duplicate series of Barcroft manometers were taken, each working flask containing 0.05M-phosphate buffer, pH 7.3, 0.005M-fumarate and the enzyme preparation. The individual additions to the flasks and their further manipulation were as follows:

Addition	Treatment
(1) None	Allowed to respire for 40 min. after equilibration. O_2 uptake measured.
(2) Pyruvate	Deproteinized after equilibration. Pyruvate content determined.
(3) Pyruvate	Allowed to respire for 40 min. after equilibration, then deproteinized. O_2 uptake and pyruvate content determined.
(4) A.N.	Allowed to respire for 40 min. after equilibration. O_2 uptake measured.
(5) A.N. + pyruvate	Deproteinized after equilibration. Pyruvate content determined.
(6) A.N. + pyruvate	Allowed to respire for 40 min. after equilibration, then deproteinized. O_2 uptake and pyruvate content determined.

In the absence of adenine nucleotide, the difference in O_2 uptake between manometers (3) and (1) gave the extra O_2 uptake due to pyruvate oxidation during the 40 min. respiration period; the difference between the pyruvate contents of flasks (2) and (3) gave the simultaneous pyruvate disappearance. Similarly, for pyruvate oxidation in the

presence of adenine nucleotide, the extra O_2 uptake was given by the difference between manometers (6) and (4), and the pyruvate disappearance was equal to the difference in pyruvate content of flasks (5) and (6). By further subtraction of these differences (as shown in Table 6), the net effect of adenine nucleotide on the O_2 uptake and pyruvate disappearance in the presence of phosphate and fumarate was determined, and the ratio of these quantities gave the O_2 /pyruvate ratio for pyruvate oxidation activated by combined phosphate-fumarate-adenine nucleotide catalysis. The results obtained in three experiments are shown in Table 6. It should be noted that the method of evaluating the effect of adenine nucleotide on the enzyme system is quite rigid since fumarate oxidation is unaffected by adenine nucleotide (Long, 1945).

Table 6. *The O_2 /pyruvate ratio for phosphate-fumarate-adenine nucleotide catalysis of pyruvate oxidation*

For experimental details, see text. The amount of dialyzed brain suspension corresponds to 333 mg. fresh tissue. Respiration period, 40 min. (A.N. = adenine nucleotide.)

Exp.	Extra O ₂ uptake (μmol.)			Pyruvate disappearance (μmol.)			Net molar O ₂ / pyruvate ratio
	A.N.		Diff.	A.N.		Diff.	
	absent	pre- sent		absent	pre- sent		
	(3)-(1)	(6)-(4)		(2)-(3)	(5)-(6)		
1	3.39	13.21	9.82	3.52	7.50	3.98	2.47
2	1.54	12.00	10.46	3.28	7.42	4.14	2.52
3	5.59	11.04	5.45	3.98	6.30	2.32	2.35
						Av.	2.45

From Table 6 emerges the important result that the O_2 /pyruvate ratio for phosphate-fumarate-adenine nucleotide catalysis of pyruvate oxidation averages 2.45; complete oxidation of pyruvate requires 2.50.

DISCUSSION

Two significant facts have emerged from the work described in the experimental part of this paper. First, it has been found that fumarate catalytically stimulates the O_2 uptake of dialyzed pigeon brain suspensions respiring in pyruvate only when adenine nucleotide is also present. Since it has already been shown (Long, 1943) that the stimulation of pyruvate oxidation by adenine nucleotide only occurs in the presence of inorganic phosphate, it follows that the combined activation must be regarded as phosphate-fumarate-adenine nucleotide catalysis. The other important observation is that fumarate is without effect on the utilization of pyruvate by this enzyme system and that its action consists solely in bringing about *complete* oxidation of that amount of pyruvate which was only *partially* oxidized in its absence.

In view of the finding that the rate of utilization of pyruvate by respiring suspensions of pigeon brain

is not increased by fumarate, the effect of the latter on pyruvate oxidation in tissues other than brain must now be considered. It has been pointed out earlier that most workers have investigated this problem using a medium containing malonate, so that their findings cannot be examined here. The few observations recorded in the literature, made in the absence of malonate, on tissues respiring in pyruvate which showed a marked increase in O_2 uptake when fumarate was added, are listed in Table 7.

of phosphate and adenine nucleotide only, the O_2 /pyruvate ratio in pigeon brain averaged 1.20; when fumarate is also present in the medium the data in the present work indicate an O_2 /pyruvate ratio of 2.50, i.e. complete oxidation. Thus the catalytic action of fumarate cannot be exerted directly on pyruvate itself since there is no change in the rate of pyruvate utilization. The conclusion to be drawn, therefore, is that fumarate is acting upon some intermediary oxidation product of pyruvate. In previous work (Long, 1943) evidence based

Table 7. *The effect of fumarate on pyruvate disappearance in animal tissues*

Tissue	No. of exp.	Fumarate conc. (M)	Pyruvate disappearance (mg./g./hr.)		% change due to fumarate	Reference
			Fumarate absent	Fumarate present		
Rabbit liver	4	0.0083	5.64	4.93	-13	Annau <i>et al.</i> (1936)
	2	0.0058	4.35	3.50	-20	
Pigeon breast muscle	1	0.0025	8.19	9.89	+21	Krebs & Eggleston (1940)
	1	0.0025	12.80	10.91	-15	
Pigeon brain	6	0.0050	4.59	3.82	-17	Present work

The results of Banga *et al.* (1939) were not presented in a form suitable for inclusion in the above table.

It will be observed from Table 7 that the data of Annau *et al.* (1936) show a consistently smaller pyruvate disappearance in the presence of fumarate than in its absence. These results for rabbit liver are in complete agreement with those obtained in the present work on pigeon brain. The two results of Krebs & Eggleston (1940) do not allow any conclusion to be reached since they are at variance with one another. The only positive evidence in favour of an increased pyruvate utilization in the presence of fumarate is to be found in the work of Banga *et al.* (1939); their data for pigeon brain, however, are offset by the results obtained in the present work.

Taken as a whole, there seems to be no clear-cut evidence in the literature for an increased pyruvate utilization accompanying fumarate catalysis of the respiration rate in any animal tissue. On the other hand, the results of Annau *et al.* (1936) on rabbit liver and those given in the present paper on pigeon brain constitute good evidence for a slightly decreased pyruvate disappearance under the conditions of fumarate catalysis. In pigeon brain, this decreased rate of disappearance of pyruvate is due to the simultaneous oxidation of fumarate to pyruvate and it seems likely that the same explanation will hold for rabbit liver. When this simultaneous reaction is taken into account there is strong evidence for the absence of any effect of fumarate on pyruvate utilization.

It was shown (Long, 1943) that, in the presence

on data obtained for the R.Q. of pyruvate oxidation indicated that 60% of the carbon of pyruvic acid was oxidized to CO_2 in the presence of phosphate and adenine nucleotide only, the remaining 40% of the carbon being converted into unknown fixed acids. It seems very probable that fumarate is acting upon these fixed acids converting them to CO_2 and water.

SUMMARY

1. Fumarate stimulates the O_2 uptake of dialyzed pigeon brain suspensions, respiring in pyruvate, only when phosphate and adenine nucleotide are also present in the medium.

2. In the presence of fumarate there is a slightly decreased rate of disappearance of pyruvate under all conditions. This is explained by the fact that fumarate is simultaneously oxidized to pyruvate, and since the amount formed in this way almost exactly balances the decreased pyruvate disappearance, it is concluded that fumarate is without effect of the rate of utilization of pyruvate.

3. Under the conditions of phosphate-fumarate-adenine nucleotide catalysis, the O_2 /pyruvate ratio was found to approach 2.50, i.e. pyruvate was oxidized completely to CO_2 and water.

4. It has been concluded from the data obtained that fumarate does not act directly on pyruvate, but on some intermediary product arising during pyruvate oxidation.

REFERENCES

- Annau, E., Banga, I., Blazso, A., Brukner, V., Laki, K., Straub, F. B. & Szent-Györgyi, A. (1936). *Hoppe-Seyl. Z.* **244**, 105.
- Annau, E., Banga, I., Gözsy, B., Huszak, S., Laki, K., Straub, F. B. & Szent-Györgyi, A. (1935). *Hoppe-Seyl. Z.* **236**, 1.
- Annau, E. & Erdős, T. (1939). *Hoppe-Seyl. Z.* **257**, 111.
- Banga, I., Ochoa, S. & Peters, R. A. (1939). *Biochem. J.* **33**, 1980.
- Gözsy, B. & Szent-Györgyi, A. (1934). *Hoppe-Seyl. Z.* **224**, 1.
- Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
- Long, C. (1942). *Biochem. J.* **36**, 807.
- Long, C. (1943). *Biochem. J.* **37**, 215.
- Long, C. (1945). *Biochem. J.* **39**, 143.
- Smyth, D. H. (1940). *Biochem. J.* **34**, 1046.
- Stare, F. J. & Baumann, C. A. (1937). *Proc. Roy. Soc. B.* **121**, 338.

The Glucuronidase Content of Animal Tissues and the Role of the Spleen in the Metabolism of Conjugated Glucuronic Acids

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It has been recognized for many years that the formation of conjugated glucuronic acids is an important result of the introduction into the body of many foreign organic compounds containing a hydroxyl or carboxyl group. That the main site of formation of these glucuronides is the liver has been established by Hemingway, Pryde & Williams (1934) using perfusion experiments and by Lipschitz & Bueding (1939) using the tissue slice technique.

The enzyme β -glucuronidase, which hydrolyzes glucuronides is found in several tissues (Masamune, 1934; Oshima, 1934, 1936; Fishman, 1938, 1939, 1940). Fishman has concluded, therefore, that the liver is not the sole site of glucuronide formation in the body. Fishman found the spleen to be a rich source of glucuronidase, the liver, ovary, uterus and kidney being relatively good sources. On feeding dogs or mice with menthol or borneol for a few days, Fishman (1940) observed that the glucuronidase content of liver, kidney and spleen increased, whereas that in the uterus remained the same and that in the ovary decreased. When oestrogenic substances (oestrone, oestradiol, etc.) were fed, the uterine glucuronidase increased but the liver glucuronidase remained unchanged (Fishman & Fishman, 1944). They also found that ovariectomy caused a decrease in uterine glucuronidase but not in the liver enzyme. Fishman (1940) concludes on the basis of his experiments that glucuronides are formed in organs other than the liver and that the uterus is the sole site of formation of the oestrogen glucuronides.

Lipschitz & Bueding (1939), on the other hand, found that liver slices were the only tissues to form significant amounts of glucuronides in the presence of menthol and borneol, the spleen being inactive and the kidney only slightly active in this respect.

The question arises, therefore, whether the glucuronidase present in tissues is concerned with the synthesis of glucuronides (Florkin, Crismer, Duchateau-Bosson & Houet (1942), found glucuronidase to synthesize bornylglucuronide *in vitro* to a small extent from borneol and glucuronic acid), or whether it is concerned only with their hydrolysis. In this connexion, it should be mentioned that glucuronides when fed to animals are hydrolyzed in the body, for Quick (1928) has shown that on feeding benzoylglucuronide, about 50% of its benzoic acid is excreted conjugated with glycine in the dog.

That liver and spleen glucuronidase may be different is indicated by the work of Talbot, Ryan & Wolfe (1943) who found that the spleen enzyme prepared according to Fishman (1938) would not hydrolyze pregandiolglucuronide in urine satisfactorily, whereas a preparation from liver would.

The work of Mitsuba (1927) suggests that the spleen may play a part in the destruction of glucuronides. This worker found that, after splenectomy, the amount of glucuronic acid excreted by rabbits and dogs in response to anthranilic acid was greater than in the intact animal. In view of the fact that Mitsuba's method for estimating glucuronic acid is now considered unreliable and consequently that his experimental findings are inconclusive, it was decided to reinvestigate this problem. At the same time, more detailed figures than were hitherto available for the glucuronidase content of rabbit and rat organs are presented.

METHODS

Enzyme extracts for assay. The animals were killed by a blow on the head and the organs dissected out, minced finely with scissors, ground with silver sand and 3 vol. of water, shaken vigorously for 1 hr. and allowed to stand