CL. METABOLISM OF NORMAL AND TUMOUR TISSUE.

IX. AMMONIA AND UREA FORMATION.

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As has been indicated in Part VIII of this series [Dickens and Greville, 1933, 1], the oxidation of protein by animal tissues may lead to different values of the respiratory quotient according to the nature of the nitrogenous end-products. The calculation of the theoretical values of R.Q. from protein oxidation is difficult, but arbitrary values showing the order of R.Q. to be expected may be derived using Loewy's [1911] figures for the analysis of meat protein and assuming the complete elimination of the protein-nitrogen as ammonia, urea or uric acid. When this is done the values obtained are approximately 0.94, 0.8 and 0.7 respectively. If, then, protein oxidation becomes an appreciable part of the total aerobic metabolism, the R.Q. may be greatly altered in a direction determined by the nature of the nitrogenous end-product, so that for the interpretation of the R.Q. it is important to establish the extent and nature of the protein oxidation and to assess its influence on the respiratory measurements.

Whilst numerous estimations of ammonia formation in isolated animal tissues have appeared, relatively few of them apply to the conditions of our experiments, and in still less has any correlation with the respiratory exchange been attempted. In the present paper we record estimations of NH_3 formation under the conditions previously employed for the classification of tissues according to their level of R.Q. in glucose-containing media [Dickens and Šimer, 1930, 2; 1931, 2]. The effect of sugar deprivation on the metabolism has already been described [Dickens and Greville, 1933, 1, 2] and we therefore include measurements of NH_3 formation in glucose-free media. As an example of the effect of other sugars we have extended the measurements to the behaviour of tissues in fructose. In all these cases simultaneous measurements of respiratory and glycolytic exchange have been carried out on the same portions of tissue as those used for the ammonia measurements. In addition, in a few selected cases the urea formation has been simultaneously estimated.

Methods.

The respiratory measurements were made by the methods of Dickens and Šimer in phosphate- [1930, 1] and in bicarbonate-Ringer solution [1931, 1]. Thin slices of tissue, prepared immediately after the death of the animal, were well rinsed by suspending in oxygenated sugar-free Ringer solution for 5–10 minutes. Equal weighed portions (usually 70–100 mg. wet weight) were then transferred to Ringer solution (1.5 to 3 cc.) contained in the manometric vessels, provided with side-bulbs containing a slight excess of 2N HCl. The initial p_{H}

of the Ringer solution was 7.4 and when glucose and fructose were added their concentration was 0.2 %. For each experiment two such vessels were used, placed side by side in the thermostat at 37°. After shaking for 10 minutes the acid was added from the bulb to one portion, which was removed from the bath after the necessary measurements of pressure change giving the CO_2 content, and the contents were used for determination of the preformed NH_3 . The respiratory experiment was continued with the other portion of tissue, which was treated similarly at the end of the experiment. The difference of ammonia contents, corrected if necessary for any difference in the dry weights of the two portions of tissue, gave the ammonia formation during the experimental time of 2 to 5 hours. A similar procedure was used for the anaerobic experiments, which were made in bicarbonate-Ringer solution saturated with nitrogen containing 5 % CO_2 .

Ammonia was estimated by the method of Stanford [1923] with the modifications recommended by Watchorn and Holmes [1927] and Bülow and Holmes [1932]. Deproteinisation, by addition of a slight further excess of $N\text{HCl}$ (0.1 cc.) and 10 % sodium tungstate (0.5 cc.), was used in some experiments; and whilst this was found advisable with some tissues, particularly with spleen with which without deproteinisation foaming may occur during distillation, its use did not ever materially affect the ammonia values obtained. Ammonia-free water and tested reagents were used, and no ammonia was allowed in the laboratory. With these precautions the results of duplicate analyses agreed well, particularly when the ammonia-formation was pronounced. For very small amounts (under 4 γ , say) the method is probably not sufficiently accurate; though provided the quantities of tissue are sufficient, as in our experiments, such experiments are of value in showing that the amount of ammonia formation is negligible in relation to the total metabolism.

Figures for "preformed" ammonia estimations are often lacking from publications on this subject. That they are frequently large, indicating a far higher ammonia content than that of the blood, for example, has been pointed out for brain-tissue by Schwarz and Dibold [1932]. Our experience confirms this; and in some of the other tissues quite high values were obtained (Table I). On the origin of this ammonia, on the extent to which it is traumatic, we express no opinion. Determinations of the ammonia contents of different slices of the same tissue, treated similarly as in our experiments, agree quite well; and nothing suggests that any uncertainty as to the "preformed" value is considerable enough to cause significant error in our measurements of the change in total ammonia.

Urea was estimated by the manometric urease method of Krebs and Henseleit [1932], which is particularly suitable for the detection of very small quantities of urea in these experiments, since its accuracy is unaffected by the presence of ammonia. Instead of soya bean we used powdered jack bean ("Arlco") with excellent results, as shown by analysis of standard urea solutions.

Results.

The data showing the extent of ammonia formation are collected in Table I, where the ammonia formation in bicarbonate- (B) or phosphate-Ringer (P) solution is given in γNH_3 per mg. dry weight of tissue. The actual amounts of NH_3 measured were usually from 5 to 15 times as great, according to the weight of tissue used. The simultaneously determined values of respiration (Q_{O_2}), R.Q., aerobic and anaerobic glycolysis ($Q_M^{O_2}$ and $Q_M^{N_2}$) are given in Table II for the same pieces of tissue as those used for ammonia estimation. Table III shows

Table I. *Ammonia formation.*

Ammonia-nitrogen, 10 ⁻³ mg. per mg. dry tissue											
Exp. No.	Medium	Time (hours)	Glucose			Fructose			No addition		
			Pre-formed	Final	Diff.	Pre-formed	Final	Diff.	Pre-formed	Final	Diff.
AEROBIC EXPERIMENTS.											
Retina (rat):											
1	P	5	3.6	3.2	-0.4	—	—	—	2.3	3.2	0.9
2	B	5	0.34	0.84	0.50	0.17	0.21	0.04	—	—	—
3	P	5	—	—	—	{ 1.1 1.5	3.0 3.0	1.9 1.5	4.7	6.4	1.7
Testis (rat):											
4	B	3	0.07	0.08	0.01	0.10	0.09	-0.01	0.32	0.72	0.40
5	B	3	0.28	0.23	-0.05	—	—	—	0.23	0.61	0.38
Kidney (rat):											
6	B	3	0.82	2.73	1.91	0.60	2.01	1.41	0.53	3.67	3.14
7	B	3	0.12	2.55	2.43	0.16	1.52	1.36	0.18	2.72	2.54
8	B	3	0.40	0.79	0.39	0.29	0.67	0.38	0.26	3.30	3.04
Spleen (rat):											
9	B	3	1.03	1.10	0.07	1.40	1.82	0.42	0.71	1.65	0.94
10	B	3	—	—	—	0.59	0.59	0.00	0.40	2.08	1.68
11	B	2.25	{ 0.33 0.36	0.64 0.63	0.31 0.27	—	—	—	0.56	2.38	1.82
Liver (rat):											
12	B	4.08	—	—	—	{ 0.18 0.13	0.20 0.22	0.02 0.09	0.23	0.14	-0.09
13	B	3	0.38	0.18	-0.20	0.42	0.27	-0.15	0.12	0.22	0.10
Embryo (chick)*:											
a 14	B	4	0.64	0.75	0.11	0.51	0.79	0.28	0.54	1.65	1.11
b 15	P	4.17	—	—	—	0.72	0.70	0.0	—	—	—
c 16	B	3.5	0.30	0.34	0.04	0.16	0.66	0.50	0.48	1.00	0.52
d 17	P	3.5	0.50	0.74	0.24	0.52	0.78	0.26	0.51	1.21	0.70
e 18	P	3	—	—	—	0.89	0.99	0.10	0.59	1.65	1.06
f 19	P	4.25	—	—	—	0.34	0.82	0.48	0.25	2.14	1.89
Embryo (rat)*:											
g 20	B	3	0.30	0.30	0.00	0.25	1.10	0.85	0.0	1.70	1.70
Yolk-sac (rat):											
21	B	3.87	0.29	0.32	0.03	0.21	0.30	0.09	—	—	—
22	B	3	—	—	—	—	—	—	0.74	0.87	0.13
23	B	3.17	0.32	0.78	0.46	—	—	—	0.47	0.71	0.24
Jensen sarcoma (rat):											
24	B	3	0.47	0.53	0.06	—	—	—	0.16	1.74	1.58
25	B	3	0.15	0.14	0.01	0.07	0.28	0.21	0.23	0.23	0.00
26	P	2.5	—	—	—	0.22	0.58	0.36	0.29	2.52	2.23
27	B	2.5	0.28	0.35	0.07	—	—	—	0.35	1.91	1.56
28	B	3	—	—	—	0.51	1.06	0.55	0.60	2.78	2.18
ANAEROBIC EXPERIMENTS.											
Kidney (rat):											
6	B	3	0.96	1.68	0.72	0.47	1.30	0.83	0.56	0.81	0.25
7	B	3	—	—	—	—	—	—	0.23	0.23	0.00
8	B	3	0.42	1.97	1.55	0.55	1.60	1.05	0.21	0.16	-0.05
30	B	2	0.36	0.26	-0.10	—	—	—	0.21	0.75	0.54
Testis (rat):											
5	B	0	0.71	—	—	—	—	—	0.49	—	—
		1	—	—	—	—	—	—	—	0.82	0.33
		3	—	0.86	0.15	—	—	—	—	1.42	0.93
Liver (rat):											
13	B	3	0.34	—	—	0.36	0.50	0.14	0.37	0.21	-0.16
12	B	4	—	—	—	—	—	—	1.0	1.10	0.10
29	B	3.33	{ 0.51 0.30	0.72 0.43	0.21 0.13	—	—	—	—	—	—
Spleen (rat):											
9	B	3	—	—	—	—	—	—	0.65	1.40	0.75
10	B	3	—	—	—	0.54	1.75	1.21	2.30	3.50	1.20
Jensen sarcoma (rat):											
24	B	3	—	—	—	—	—	—	0.35	0.87	0.52
25	B	3	1.01	0.71	-0.30	0.40	0.89	0.49	0.57	0.41	-0.16
26	B	2.5	—	—	—	—	—	—	0.28	0.86	0.58

* Embryo dry weights: a, 6.0; b, 5.1; c, 7.6; d, 7.6; e, 5.8; f, 8.3; g, 6.8 mg.

Table II. *Respiration, respiratory quotient and glycolysis.*

Exp. No.	Medium	Time (hours)	Glucose				Fructose				No addition			
			R.Q.	Q _{O₂}	Q _M ^{O₂}	Q _M ^{N₂}	R.Q.	Q _{O₂}	Q _M ^{O₂}	Q _M ^{N₂}	R.Q.	Q _{O₂}	Q _M ^{O₂}	Q _M ^{N₂}
Retina:														
1	P	5	—	24.5	—	—	—	—	—	—	—	3.0	—	—
2	B	5	0.94	20.3	22.5	—	0.94	17.2	0.3	—	—	—	—	—
3	P	5	—	—	—	—	1.02 0.99	14.6 16.2	—	—	—	4.5	—	—
Testis:														
4	B	3	0.88	10.2	6.8	—	0.82	8.5	2.1	—	0.82	4.8	1.8	—
5	B	3	0.93	6.9	4.2	—	—	—	—	—	0.76	3.1	0.9	—
Kidney:														
6	B	3	0.87	24.0	-0.9	4.8	0.92	24.6	0.0	1.3	0.84	17.3	-1.7	1.1
7	B	3	0.84	19.3	0.0	1.8	0.91	27.6	0.1	—	0.80	19.0	-1.1	2.8
8	B	3	0.87	22.2	2.0	7.6	0.89	24.9	0.2	2.8	0.79	18.9	-0.6	1.3
30	B	2	—	—	—	3.7	—	—	—	—	—	—	—	1.3
Spleen:														
9	B	3	0.92	10.8	4.8	—	0.81	10.5	3.6	—	0.83	8.9	0.3	6.6
10	B	3	0.89	11.0	5.3	—	0.85	12.7	4.0	5.6	0.83	9.0	0.6	4.4
11	B	2.25	0.85 0.86	17.0 14.6	6.7 6.5	—	—	—	—	—	—	—	—	—
Liver:														
12	B	4.08	—	—	—	—	0.79 0.77 0.78	8.5 7.4 7.0	2.9 3.5 3.0	4.0 — 3.6	0.70	7.8	2.0	1.4
13	B	3	0.67	7.3	2.8	1.7	—	—	—	—	0.58	8.6	2.1	1.5
Chick embryo:														
14	B	4	0.95	10.9	4.5	—	0.94	8.1	-0.6	—	0.91	8.0	-0.7	—
15	P	4.17	—	13.3	—	—	—	12.1	—	—	—	11.1	—	—
16	B	3.5	1.02	9.1	2.4	—	0.89	8.5	0.0	—	0.87	7.5	0.0	—
17	P	3.5	1.02	10.3	—	—	0.95	9.1	—	—	0.95	9.0	—	—
18	P	3	—	—	—	—	0.96	9.3	—	—	0.87	9.2	—	—
19	P	4.25	—	—	—	—	0.87	9.6	—	—	0.89	8.3	—	—
Rat embryo:														
20	B	3	0.97	11.1	5.2	—	0.95	11.4	0.0	—	0.78	9.2	0.7	—
Rat yolk-sac:														
21	B	3.9	—	(11.0)	—	—	0.98	11.0	1.1	—	0.91	11.4	0.3	—
22	B	3	—	—	—	—	—	—	—	—	0.80	11.0	1.1	—
23	B	3.17	1.05	10.3	2.4	—	—	—	—	—	0.92	8.9	1.1	—
Jensen rat sarcoma:														
24	B	3	0.81	13.6	11.1	—	—	—	—	—	0.67	10.4	0.4	1.2
25	B	3	—	—	—	21.2	0.76	11.3	6.6	10.7	0.73	12.4	0.4	1.8
26	P	2.5	0.85	10.6	—	—	0.87	13.1	—	—	0.81	12.8	—	1.4
27	B	2.5	0.83	9.3	9.3	—	—	—	—	—	0.73	9.1	0.7	—
28	B	3	—	—	—	—	0.81	14.1	5.6	—	0.78	12.6	0.1	—

Table III. *Urea formation*.*

Exp. No.	Medium	Time (hours)	Glucose				Fructose				No addition			
			R.Q.	Q_{O_2}	$Q_M^{O_2}$	Q_{urea}	R.Q.	Q_{O_2}	$Q_M^{O_2}$	Q_{urea}	R.Q.	Q_{O_2}	$Q_M^{O_2}$	Q_{urea}
Chick embryo (5th day):														
31	B	4	—	9.8	—	+0.1	0.85	10.4	0.4	0.0	0.84	8.7	−0.3	0.0
32	P	4	—	—	—	—	0.96	9.7	—	0.0	0.94	8.2	—	+0.1
Rat yolk-sac:														
33	B	4	1.04	12.0	2.3	+0.1	—	—	—	—	0.94	11.8	−0.4	0.0
											0.94	11.9	0.0	—
Jensen rat sarcoma:														
27	B	2.5	0.83	9.3	9.3	+0.2	—	—	—	—	0.73	9.1	0.7	0.0
Rat liver:														
34	B	3	0.60	7.8	3.5	+0.2	0.70	9.0	3.4	+0.1	0.66	8.3	2.4	+0.3

* $Q_{urea} = \frac{\text{mm.}^3 \text{ urea-CO}_2}{\text{mg. (dry) tissue} \times \text{hrs}}$. (To express as mg. urea per mg. per hour, multiply by 0.269×10^{-3} .)

the results of simultaneously made estimations of urea. In this case the values of "preformed" urea are omitted as they were in all cases negligible [*cf.* Krebs and Henseleit, 1932].

From the figures given in Table III it is evident that in none of the cases examined (rat liver, embryo, yolk-sac, Jensen rat sarcoma) was the urea formation appreciable when compared with the total metabolism, under the conditions of our experiments. The effect on the R.Q. of retention of carbon as urea to the extent indicated in Table III would be hardly detectable. Naturally, these observations apply only to washed tissues in ordinary Ringer solutions. Under special conditions, as in the presence of fairly high concentrations of ammonia, the urea formation by isolated liver tissue may be very large, as Krebs and Henseleit have shown. It should be noted that these authors found that rat brain, retina, kidney, spleen, testis and yolk-sac do not produce measurable amounts of urea, even under conditions which are optimum for urea formation by liver. Taking our results in conjunction with these, we may probably neglect the possibility of urea formation by the tissues discussed in this paper.

Relationship of NH_3 formation and respiration. In order to correlate the observed values of respiration (Q_{O_2}) with the ammonia formation it is convenient to calculate the latter in similar units to the Warburg units for respiration: Q_{NH_3} is then expressed in mm.³ NH_3 (at 0° and 760 mm.) formed per mg. dry weight of tissue per hour (10^{-3} mg. $NH_3 = 1.32$ mm.³). These values, and their means, have been collected in Table IV.

These figures, taken in conjunction with the respiratory measurements already given, give an idea of the extent to which protein oxidation leading to ammonia may contribute to the total metabolism. Taking the figures used by Loewy [1911] for the analysis of a typical protein, and assuming complete elimination of all N as ammonia with complete oxidation of the remainder of the molecule, the calculation of R.Q. for pure protein oxidation is as follows:

100 g. protein	C 58.4	H 7.3	O 22.7	N 16.65	S 1.02
Deduct for NH_3		3.6		16.65	
" S-oxidation			1.5		1.02
" intramolecular H_2O		2.65	21.2		
Residue for oxidation	C 58.4	H 1.05			
Requires: O_2	155.8 + 8.4 + 1.5 = 165.7 g.				
Produces: CO_2			214.2 g.		
	R.Q. = $\frac{109}{116} = 0.94$.				

Oxidation of 1 mg. protein thus requires 1160 mm.³ oxygen and produces 1090 mm.³ carbon dioxide together with 0.202 mg. NH_3 (= 266 mm.³). Hence the ratio

$$\frac{Q_{O_2}}{Q_{NH_3}} = \frac{\text{mols. oxygen consumed}}{\text{mols. ammonia produced}} = \frac{1160}{266} = 4.4 \text{ approx.}$$

for the complete oxidation of this protein to NH_3 , CO_2 , H_2O and SO_3 . Since the tissue proteins vary somewhat in composition and it is uncertain how far the oxidation follows the above course, it is clear that this value may only be used as an approximation, but as such it is a useful guide to the probable amount of ammonia-producing protein oxidation. In the right-hand columns of Table IV are given the individual and mean values of the ratio Q_{O_2}/Q_{NH_3} : in the calculation of the latter the mean Q_{O_2} of all experiments with the given tissue (Table II) has been divided by the mean Q_{NH_3} .

Inspection of these figures shows that the ammonia formation is almost always greatest when no sugar is supplied to the tissue. In sugar-free media

the amount of protein oxidation, if this be indicated by the Q_{O_2}/Q_{NH_3} ratio, may rise to about one-half of the total respiration (retina, kidney, spleen, Jensen rat sarcoma). In the ammonia-producing tissues, with the exception of kidney,

Table IV. *Relation of ammonia formation to respiration.*

Tissue	Exp. No.	Q_{NH_3}			$\frac{\text{mols. } O_2}{\text{mols. } NH_3} (= Q_{O_2}/Q_{NH_3})$		
		Glucose	Fructose	No addition	Glucose	Fructose	No addition
Retina	1	-0.13	—	0.29	(-190)	—	10.3
	2	0.16	0.01	—	127	1700	—
	3	—	{ 0.60 0.48	0.55	—	{ 24 34	8.2
	Mean*	0.02	0.28	0.42	Large	58	9
Testis	4	0.005	-0.005	0.21	2000	(-1700)	23
	5	-0.02	—	0.18	(-350)	—	17
	Mean	(-0.01)	(-0.005)	0.20	Large	Large	20
Kidney	6	1.02	0.75	1.67	23.5	32.8	10.3
	7	1.29	0.73	1.35	15.0	37.8	14.0
	8	0.21	0.20	1.62	106	124	11.7
	Mean	0.84	0.56	1.55	26	46	12
Spleen	9	0.04	0.22	0.50	270	48	17.8
	10	—	0.00	0.90	—	Large	10.0
	11	{ 0.22 0.19	—	1.29	{ 77 77	—	7.0
	Mean	0.12	0.11	0.90	104	105	10
Liver	12	—	{ 0.008 0.035	-0.035	—	{ 106 212	(-220)
	13	-0.11	-0.08	0.053	(-66)	(-87)	162
	Mean	-0.11	-0.01	0.01	(-ve)	(-ve)	(Large)
Chick embryo	14	0.044	0.11	0.44	250	74	18
	15	—	0.00	—	—	Large	—
	16	0.018	0.23	0.24	510	37	31
	17	0.11	0.12	0.32	94	76	28
	18	—	0.05	0.57	—	184	16
	19	—	0.18	0.71	—	53	11.7
	Mean	0.06	0.12	0.46	190	79	20
Rat embryo	20	0.00	0.45	0.91	Large	25.4	10.1
Rat yolk-sac	21	0.012	0.04	—	920	270	—
	22	—	—	0.07	—	—	160
	23	0.23	—	0.12	45	—	75
	Mean	0.12	0.04	0.10	89	270	104
Jensen rat sarcoma	24	0.032	—	0.84	425	—	12.4
	25	0.005	0.11	0.00	2400	103	(Large)
	26	—	0.23	1.42	—	57	9.0
	27	0.04	—	1.00	230	—	9.1
	28	—	0.29	1.16	—	49	10.9
	Mean	0.026	0.21	0.88	430	61	13

* The mean values of $\frac{\text{mols. } O_2}{\text{mols. } NH_3}$ given are the mean values of Q_{O_2} divided by the mean values of Q_{NH_3} .

the "protein-sparing" action of added sugar is apparent; for in the presence of glucose the amount of oxidation of nitrogenous bodies leading to ammonia formation is certainly small. If reckoned as protein oxidation it usually amounts to less than 5 % of the total oxygen uptake. "Protein-sparing" is also seen to

occur in the presence of fructose; and our experiments provide an interesting demonstration that the phenomenon of "protein-sparing" by carbohydrates applies to a wide variety of tissues and persists with isolated tissues *in vitro*.

The extent to which ammonia formation occurs is so different with different tissues that each must be considered separately. Under our conditions washed liver tissue produces barely appreciable quantities of ammonia, and a slight ammonia consumption sometimes occurs. It is highly probable that the latter is due to urea formation, which, as Krebs and Henseleit [1932] showed, proceeds more readily from ammonia in slices of surviving liver tissue than in any other tissue examined by them. The experiment (No. 34) in Table III gives an idea of the magnitude of urea formation in washed liver. It is roughly of the same order as the ammonia figures for the same tissue, that is to say too small for accurate measurement and negligible in its contribution to the total metabolism. Hence the low R.Q. found with washed liver slices is not due to urea formation, and, unless uric acid or some unconsidered nitrogen compound be formed, the liver does not derive its energy from oxidation of its own protein. Since isolated liver tissue does not apparently oxidise glucose from the medium to any great extent [Dickens and Greville, 1933, 1], it seems probable that liver uses a mixed oxidation of its own fat and carbohydrate (glycogen) reserves. The value of the R.Q. is in accordance with this view [Dickens and Šimer, 1930, 2; 1931, 2]. In the presence of fructose liver may show a large glycolysis, and the R.Q. is considerably raised. This shows that the power to oxidise carbohydrate is definitely present in liver tissue [Dickens and Greville, 1933, 1], although carbohydrate oxidation has never been observed to cover the whole respiration. Possibly by the use of strong fructose solutions the percentage carbohydrate oxidation could be further increased: this is being investigated.

Unlike liver, kidney shows a strong ammonia formation in all experiments in sugar-free media, only partially removed by addition of glucose or fructose. This is of particular interest since the kidney is considered by many workers to be the seat of nearly all the ammonia formation in the body [see Krebs, 1932]. Warburg, Posener and Negelein [1924], however, on the basis of the temperature coefficient, considered that ammonia elimination by isolated rat's kidney was not a chemical reaction but a mechanical washing-out of preformed ammonia from the tissue. They further stated that sugar did not affect the ammonia production. The evidence, however, was not convincing and was greatly weakened by the demonstration of Patey and Holmes [1929] and of Holmes and Patey [1930] that the ammonia production was much less under anaerobic conditions. These authors, however, using the differential Barcroft apparatus and chopped kidney were unable to show that deamination of added amino-acid (glycine) was accompanied by a comparable extra oxygen uptake; but Krebs [1932] has since shown that with tissue slices such an increase regularly occurs and has demonstrated beyond doubt an oxido-deaminase in kidney and its extracts. Holmes and Patey, however, were able to show that, contrary to Warburg, Posener and Negelein's statement, the aerobic ammonia production of kidney from its own nitrogen compounds was checked by the addition of glucose, whilst the anaerobic conversion was unaffected by sugar. The ammonia formation from added amino-acids was more sensitive to cyanide than that occurring without substrate, but like the anaerobic breakdown was unaffected by glucose. They concluded that two aerobic and one anaerobic mechanisms were at work, all resulting in ammonia production, and suggested that at least part of the anaerobic formation was from deamination of adenylic acid [cf. Embden and Schumacher, 1929].

Our observations on kidney slices support the results of Holmes and Patey, inasmuch as the ammonia formation from the tissue's own N compounds is diminished somewhat by addition of glucose, and more markedly by addition of fructose, which is more readily oxidised than glucose by the kidney; and also in that the anaerobic formation of ammonia (Table I) without added sugar is far less than the aerobic formation. With sugar, however, the difference is much less marked, though the anaerobic results are here so variable that our few measurements can lead to no definite opinion. From the comparison of the aerobic and anaerobic ammonia formation without added sugar, from the effect of sugar, particularly fructose, in increasing the R.Q. and the total respiration and at the same time lowering the ammonia formation, we see no reason to doubt that the ammonia production thus checked is due to oxidative deamination, presumably of protein, though possibly in part of amino-acids or of other protein breakdown products existing preformed in the tissue. The Q_{O_2}/Q_{NH_3} ratio for kidney (Table IV) suggests that some 30 % of the oxygen uptake of kidney tissue without sugar may be due to protein oxidation leading to ammonia. Fructose depresses this part of the oxidation to less than one-tenth of the total. Glucose has less effect, and kidney is the one tissue yet studied in which a fairly large part (perhaps up to a quarter) of the total respiration in glucose may be due to oxidation of protein or protein derivatives. Since Holmes and Patey found that the ammonia formation by kidney from added amino-acids is not affected by glucose addition, and since kidney is the only tissue which readily deaminates added amino-acids under aerobic conditions [Krebs, 1932] and is the only tissue which produces large quantities of ammonia during its respiration in glucose, it seems possible that it is the oxidative deamination of preformed amino-acids in the tissue which may be, at any rate in part, responsible for the persistence of aerobic ammonia production in the presence of glucose. Finally, this oxidation of N compounds leading to ammonia gives a possible explanation of the anomalously high R.Q. of kidney in glucose in comparison with its ability to convert glucose into lactic acid [Dickens and Šimer, 1930, 2].

The nervous tissues, brain and retina, having normally an almost pure carbohydrate oxidation, share the property of being dependent on an external supply of sugar for the continuance of their respiration [Loebel, 1925; Dickens and Greville, 1933, 1]. The effect of glucose and lactic acid on the ammonia formation of brain has been fully investigated by numerous authors [Warburg, Posener and Negelein, 1924; Loebel, 1925; Watchorn and Holmes, 1927; Bülow and Holmes, 1932], and we have not therefore included any of our observations in this paper. The ammonia formation of brain may be lowered somewhat in the presence of oxidisable metabolites (sugar, lactic acid), but frequently the absolute amount of ammonia produced is not markedly affected, as is seen from the data of Loebel [1925]. It is mainly the ratio of oxygen respired to ammonia produced that is affected in these tissues, this being lowered in absence of sugar to a value indicating that a considerable part of the residual oxygen uptake may be due to protein oxidation. This, therefore, is a somewhat special case. The total respiration is so dependent on the presence of sugar that it falls to one-third or less in its absence. At the same time the actual ammonia formation is hardly affected. An illusion of "protein-sparing" is thus produced, in that the protein fraction of the respiration is lessened by the presence of sugar. This effect is much less in evidence in most of the other, non-nervous, tissues examined, since their respiration is less affected by the presence or absence of sugar.

The behaviour of retina closely resembles that of brain. Both tissues have a fairly large preformed ammonia content. Our experiments with retina suggest

some decrease of ammonia production in presence of sugar, but here also an illusion of great protein-sparing is produced. The value of the ratio Q_{O_2}/Q_{NH_3} suggests high protein oxidation in absence of substrate, the protein oxidation becoming relatively much less in presence of either glucose or fructose, both of which support the respiration of retina [Dickens and Greville, 1933, 1]. It is hardly practicable to measure the R.Q. of retina without added substrate; but that of brain (rat, rabbit) in our experience remains near unity under these conditions in conformity with the view that protein is being oxidised to give ammonia. As a result of one experiment Loebel [1925] shows a fall of R.Q. to 0.86, but we find that it is unusual to encounter such a marked decrease.

Among tissues with less marked glycolytic and sugar-oxidising ability, testis shows a point of resemblance to brain in that its respiration falls considerably in absence of sugar. Some indication of a protein-sparing action of the sugars is given by the fall in ammonia excretion when the tissue is supplied with either glucose or fructose (Table IV). The anaerobic ammonia formation may, however, be also surprisingly large even in the presence of sugars, making interpretation of the results difficult. It is clear from the ammonia figures, however, that the metabolism in glucose or fructose does not consist to any appreciable extent of oxidation of protein to ammonia; and support is given to the view that fructose is oxidised by this tissue [Dickens and Greville, 1933, 1].

From Table IV it is seen that spleen has a high ability to form ammonia from its own N compounds, or possibly to some extent from those of the blood which invariably accompanies this tissue into the manometric vessels, even after careful rinsing. Here both glucose and fructose reduce to a low level the high aerobic ammonia elimination found in the absence of these sugars. This supports the R.Q. measurements in indicating an oxidation of glucose by this tissue and renders it probable that fructose is also oxidised to some extent even though the R.Q. is apparently not increased by its presence.

The embryonic tissues studied were the rat yolk-sac and the embryos of chick and rabbit. In the first-named tissue the ammonia formation was low in all cases, both in presence and absence of added glucose or fructose. There is therefore little oxidation of protein resulting in ammonia formation by rat yolk-sac under any of these conditions. As Table III shows, the urea formation is also negligible. Since in the absence of added substrate the respiration does not fall significantly, and the R.Q. remains at a relatively high level, it seems probable that the metabolism of the yolk-sac may be adequately supported by its own reserves of foodstuff, whose nature is not known [Dickens and Greville, 1933, 1].

The chick embryo resembles the rat yolk-sac in the persistence of the respiration at a nearly unchanged level in the absence of glucose or fructose. On the other hand, the ammonia estimations (Table IV) show that in this tissue in absence of sugar the ammonia formation is well-marked and may indicate a fairly extensive change of metabolism by the embryo to a protein oxidation, which is checked by addition of either glucose or fructose, both in phosphate and bicarbonate media. It thus appears that the embryo has at this age a considerable ability to turn to protein as a foodstuff, but only in the absence of oxidisable carbohydrates. It should be mentioned, however, that Needham [1932] considers that about 20 % of the respiration of the chick embryo in glucose is due to protein oxidation. The decreased ammonia production in the presence of fructose indicates that this sugar can diffuse into the embryo, and that its failure to cause lactic acid production in the embryo [Dickens and Greville, 1932] is not due to impermeability. Urea formation by the chick

embryo of 4–5 days' incubation is negligible under our conditions (Table III). The rat embryo of 13–14 days' gestation shows, like the chick embryo, a large ammonia production in absence of sugar, reduced by the addition of glucose or fructose.

The tumour tissue examined—Jensen rat sarcoma—presents several points of similarity to the embryonic tissues. Table IV shows that the ammonia formation in absence of substrate is large, both in phosphate and bicarbonate media. Since the aerobic ammonia production is much greater than the anaerobic, is practically abolished in glucose and is greatly reduced in fructose, we believe that the evidence is in favour of an oxidation of protein which may constitute up to 50 % of the total respiration in absence of sugar, whilst in the presence of sugar the protein oxidation falls to a low level. In all probability both glucose and fructose are oxidised by this tumour; but from the low value of the R.Q., and the fact that the R.Q. and the respiration are hardly affected by withdrawal of sugar, we infer that complete oxidation of carbohydrate accounts for only part of the respiration of the tumour cell. Under our experimental conditions, the urea formation by Jensen rat sarcoma was insignificant and quite unable to account for the characteristically low value of the R.Q.

Oxidative deamination of nitrogen compounds in the tissue can strictly be assumed only when the aerobic ammonia production far exceeds the anaerobic. This qualification has been kept in mind throughout, but it may be unduly strict. For the deamination observed by us with certain tissues, particularly spleen, under anaerobic conditions may possibly also be oxidative, at least in part, owing to the presence of hydrogen acceptors in the tissue; so that the existence of a large anaerobic deamination does not necessarily mean that the aerobic ammonia formation is not indicative of the oxidation of nitrogenous bodies.

Oxidative ammonia production may be due to the oxidation of nitrogenous bodies other than protein. Even if it is due to protein oxidation, this may not be complete. In either case, some of our conclusions are quantitatively altered. But the answer to the point which we set out to test is independent of these considerations: in a glucose medium the oxidation of nitrogenous bodies by all the tissues examined, except kidney, is so small as to have no material influence on the respiratory quotient. This is true in so far as can be judged from ammonia and urea formation. The possibility of protein oxidation under these conditions leading to the production of uric acid or other nitrogenous bodies has yet to be tested.

SUMMARY.

1. In the presence of glucose or fructose, and also in absence of added substrate, the ammonia formation by various washed animal tissues has been compared with the respiration and respiratory quotient, observations of all three quantities being simultaneously made on the same slice of tissue.

2. Little ammonia or urea was formed under our conditions by rat liver or yolk-sac either in presence or absence of sugar. Since the respiration and R.Q. of these tissues are not greatly altered by the presence of added sugar, it seems possible that their metabolism may be adequately supported by their foodstuff reserves, which in the yolk-sac are of unknown nature.

3. With kidney, spleen, Jensen rat sarcoma and embryo (chick, rat) the aerobic ammonia formation was large in absence of sugar, but was arrested by glucose, except with kidney, where it persisted at a reduced but still large value.

4. In all cases where glucose reduced the ammonia production, fructose had a similar effect. In kidney, fructose is more readily oxidised than glucose, and correspondingly it reduces the ammonia production to a greater extent.

5. In brain, retina and testis the amount of ammonia produced was small but in absence of glucose or fructose became large relative to the respiration, because of the large fall in the latter.

6. The "protein-sparing" action of glucose and fructose has thus been demonstrated for a variety of animal tissues, but with brain and retina it may be largely illusory.

7. Anaerobic ammonia elimination under the same conditions as in the aerobic experiments may be large, as in spleen and testis, but most often it is very much less than the aerobic production.

8. Urea formation by 5-day chick embryo and by Jensen rat sarcoma is so small that its effect on R.Q. measurements under our conditions can be neglected.

9. In general, in so far as can be judged from ammonia and urea formation, the assumption made in Part II of this series, that in a glucose medium the protein oxidation is so small as to have no material influence on the R.Q., has received experimental justification. It is probable that, of the tissues studied, only in kidney does the oxidation of nitrogenous bodies cover any large part of the total oxidations under the conditions of these experiments. The relatively high value of R.Q. shown by kidney may be due in part to the oxidation of protein by this tissue.

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