SUMMARY

1. A study has been made of the reaction between triphenyltetrazolium bromide and various reducing agents. Conditions for optimal production of formazan by this reaction were established.

2. Triphenylformazan has been prepared and

its absorption and solubility characteristics determined.

3. Direct colorimetric methods for the estimation of blood sugar, glucose, lactose, cysteine and ascorbic acid based on the use of tetrazolium are described.

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The Nature and Mechanism of Action of the Enzymes Responsible for Tissue Respiration during Aseptic Autolysis

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It is known that tissues, isolated from organisms and maintained under aseptic conditions, undergo processes of autolysis, characterized by progressive hydrolysis of proteins brought about by the tissue cathepsins. The mechanism of catheptic action has been studied by several workers, and it has been shown that the activity of the cathepsins is stimulated by acids, thiols and cyanide. Less attention has been devoted to the action of other enzymes during autolysis. Edlbacher & Koller (1934) showed that necrotic tissue from Jensen's sarcoma is rich in arginase, and Bayerle (1936) extended the observation to necrotic non-cancerous tissues. Michelazzi (1947) studied the behaviour of the oxidative metabolism of tissues in conditions of aseptic autolysis, and obtained evidence that homogenates of guinea pig liver, kidney and brain can consume small amounts of oxygen even many days after the start of aseptic autolysis in the organs. Since boiling for 20 min. abolished the respiratory activity, Michelazzi concluded that this must be due to the persistence of certain oxidizing enzymes. He observed nevertheless that fumarate, lactate, aspartate, DL-alanine, phenylalanine and ethanol are not attacked by autolysing tissues and that cytochrome oxidase is completely inactive. The present work is also concerned with oxidative metabolism of the tissues in conditions of aseptic autolysis.

EXPERIMENTAL

Conditions of autolysis. Liver and kidney of guinea pigs, mice and rats were used. The organs were removed under sterile conditions from the bled animals and were placed in sterile vessels containing 30 ml. 0.067 M-acetate buffer (pH 4.5), which was then covered to a depth of 1 cm. with toluene. The vessels were closed with paraffined glass stoppers and were placed in a thermostat at 37.5° for periods of from 20 to 50 days. At the desired times the vessels were opened, the toluene removed, and the organs washed with 0.067 M-phosphate buffer (pH 7.4) blotted with filter paper, and weighed.

Homogenates were made by prolonged grinding in a mortar, the amounts of tissue used in each experiment being such that 0.5 g. of liver or 0.25 g. of kidney was contained in 3 ml. of the suspension. Controls for sterility were made by inoculation into broth, any contaminated specimens being discarded.

Measurement of respiration. Residual respiration was estimated by the Warburg technique over a period of 60 min. at 38° in 0.067 M-phosphate buffer (pH 7.4).

Succinoxidase activity was determined by the method of Schneider & Potter (1943), and lactate dehydrogenase by the method of Green & Brosteaux (1936): α -glycerophosphate dehydrogenase was studied by measuring the extra O_2 uptake in the presence of 0.2 ml. 0.2 M-sodium α -glycerophosphate (Hofmann-LaRoche). D- and L-Amino-acid oxidase activities were determined by the procedure of Rodney & Garner (1938), 0.2 ml. of DL- and L(+)-alanine (0.2 M) being used as substrates.

Monoamine oxidase activity was followed by measuring the extra O_2 uptake in the presence of 0.2 ml. of 0.25M-tyramine hydrochloride or of 0.25M-adrenaline hydrochloride (both from Hofmann-LaRoche), 0.1 ml. of 0.2M-KCN being added to each flask; control flasks to allow for the autoxidation of tyramine and adrenaline were also included.

Inhibitors. NaCN and NaF, each in a final concentration of 0.05M. Urea and hydrazine hydrate in final concentrations of 3 and 0.01M, respectively.

sec.-Octyl alcohol, 0.05 ml./Warburg flask.

Preparation of monoamine oxidase. The preparation, obtained from calf liver by the method of Kohn (1937), gave a Q_{O_2} = 12.6 μ l./mg. dry wt./hr. with tyramine as substrate. Storage at 0° for 7 days caused no significant loss of activity. The specificity of the purified enzyme was tested against the following substrates, none of which was attacked: DL-alanine, L(+)-alanine, sodium succinate, sodium lactate, sodium fumarate, sodium α -glycerophosphate, histamine hydrochloride.

Detection of amines. Amines were detected in the tissue autolysates by measuring the extra O_2 consumption induced by incubating samples of the homogenates with the purified calf-liver monoamine oxidase preparation.

Ammonia production was measured by the procedure used by Thompson & Tickner (1949).

Hydrogen peroxide production during the oxidation of tyramine was demonstrated (1) by observing the secondary oxidation of ethanol to acetaldehyde (Keilin & Hartree, 1936; Kohn, 1937), 1 mg. of ethanol being placed in the side arms of the Warburg flasks, and tipped into the main compartment 30 min. after the first reading; (2) by the oxidation of haemoglobin to methaemoglobin (Bernheim & Michel, 1937) by the following technique: at the end of the measurement of O_2 uptake in the presence of tyramine, the contents of the Warburg flasks were filtered; to the filtrate was added 0.5 ml. of a very dilute haemoglobin solution (obtained by lysis of guinea pig erythrocytes), and the mixture was allowed to stand for 2-4 hr.; in a second flask 0.5 ml. of the haemoglobin solution was added to 3 ml. 0.067M-phosphate buffer (pH 7.4) as a control. The production of a brown colour in the first flask was accepted as proof of the presence of traces of H_2O_2 .

Aldehyde production by the autolysing tissues was detected by adding to the homogenates a purified xanthine oxidase preparation, previously placed in the side arm of the Warburg flasks, and by measuring for 60 min. the extra O_2 uptake. These experiments were carried out without cyanide, which strongly inhibits xanthine oxidase. The xanthine oxidase was prepared from cream from unpasteurized milk by the method described by Ball (1939). Its activity was tested by studying the oxidation of acetaldehyde at 38° and at pH 7.5.

RESULTS

Residual respiration of autolysing tissues

The results of Michelazzi (1947), showing that homogenates of autolysing liver and kidney take up small quantities of O_2 even many days after the beginning of autolysis, have been confirmed (see Table 1).

In Table 1, since the observed O_2 uptakes were small in relation to the amount of tissue employed,

they are expressed as μ l. O_2 /hr. rather than as Q_{O_2} values.

Table 1. *Residual oxygen uptake of liver and kidney homogenates during aseptic autolysis*

(Values are expressed as μ l. O_2 /hr. Gaseous environment, air. Temp., 38°.)

No. of exp.	Animal	Time of autolysis (days)	O_2 consumed in 1 hr. (μ l.)
Liver			
1	Guinea pig	15	30.7
1	Guinea pig	17	30.6
2	Guinea pig	18	27.6
1	Guinea pig	20	27.5
3	Guinea pig	23	34.4
1	Guinea pig	25	20.4
2	Guinea pig	30	0.8
1	Guinea pig	40	0
1	Guinea pig	50	0
2	Rat	15	32.3
2	Rat	20	21.8
2	Rat	22	28.6
4	Rat	25	25.6
2	Rat	30	11.0
1	Rat	40	0
2	Mouse	20	12.4
2	Mouse	25	10.6
Kidney			
1	Guinea pig	17	20.5
3	Guinea pig	20	20.0
1	Guinea pig	25	4.2
1	Guinea pig	30	4.1
1	Guinea pig	40	0
1	Rat	15	30.5
2	Rat	20	21.0
4	Rat	25	11.5
1	Rat	26	10.3
2	Rat	30	2.7
1	Rat	50	0

Residual O_2 uptake is always small, being maximal in the first days of autolysis and then progressively decreasing. A small quantity of O_2 is consumed even after 20-30 days' autolysis; in course of time the residual respiration is completely suppressed. Residual O_2 uptake is generally higher in the liver than in the kidney; no large differences exist between guinea pig, rat and mouse in the values for liver, although those obtained with rat kidney are generally lower than those with guinea pig kidney.

Marked quantitative differences were observed among the various experiments as had also been noticed in Michelazzi's experiments.

The residual respiration of guinea pig liver was abolished completely by boiling for 2 min., and was markedly decreased by heating at 60° for 10 min.

Influence of pH on residual respiration

Measurements of residual respiration were carried out at pH 6.8, 7.0, 7.4, 7.6 and 7.8. It was found that the optimum pH for residual O_2 uptake of these homogenates of autolysing tissue is 7.4.

Table 2. *Influence of oxygen tension on residual respiration of autolysing tissue homogenates*

Animal	Organ	Time of autolysis (days)	O ₂ consumed (μl.)		Increase (%)
			In air	In 100% O ₂	
Guinea pig	Liver	20	57.4	69.2	20.5
Guinea pig	Liver	21	25.0	34.1	36.2
Guinea pig	Liver	22	16.0	26.5	54.9
Guinea pig	Liver	22	15.5	21.8	39.8
Rat	Liver	22	7.6	14.3	88.3
Rat	Kidney	22	5.3	8.6	63.5

Table 3. *Influence of some inhibitors on residual respiration of autolysing tissue homogenates*

Animal	Organ	O ₂ consumed (μl./hr.)	With KCN		With NaF		With sec.-octyl alcohol	
			O ₂ consumed (μl./hr.)	Inhibition (%)	O ₂ consumed (μl./hr.)	Inhibition (%)	O ₂ consumed (μl./hr.)	Inhibition (%)
Guinea pig	Liver	27.5	25.7	6.29	26.4	3.89	0	100
Guinea pig	Liver	29.0	26.3	9.24	29.1	0	3.5	87.96
Guinea pig	Kidney	16.4	15.6	5.05	15.9	3.16	1.1	87.51
Rat	Liver	21.8	21.1	3.48	21.6	1.00	2.3	86.84
Rat	Kidney	20.5	20.0	2.48	19.3	6.09	3.6	82.46

Influence of oxygen tension on residual respiration

The O₂ uptakes of the homogenates in air and in 100% O₂ were compared. The results are summarized in Table 2.

From Table 2 it can be seen that pure O₂ increases the rate of residual respiration of autolysing tissue homogenates. The actual increase is variable, ranging from 36 to 88%.

Action of inhibitors on residual respiration

The results obtained with certain enzyme inhibitors are shown in Table 3. It was found that cyanide and fluoride exert only a very weak effect on the residual O₂ uptake, which is, however, strongly inhibited by sec.-octyl alcohol. The weak inhibitory action of cyanide confirms the complete ineffectiveness of cytochrome oxidase in the autolysing tissues (Michelazzi, 1947) and excludes the possibility of residual O₂ uptake being due to the activity of oxidases acting through the cytochrome-cytochrome oxidase system.

Oxidative activity of autolytic tissues upon certain substrates

The autolysing tissues were tested for content of various enzymes; succinoxidase (acting through the cytochrome-cytochrome oxidase systems), lactic dehydrogenase, α-glycerophosphate dehydrogenase and D- and L-amino-acid oxidases. None of these was detected.

Since none of these enzymes maintains its activity in the autolytic tissues, it seemed likely that the enzyme responsible for the residual O₂ uptake in autolysing tissues belongs to the group of oxidases which can directly transfer hydrogen to atmospheric O₂.

Among this group is monoamine oxidase discovered by Hare (1928) and fully described by Pugh & Quastel (1937), Kohn (1937), and Blaschko, Richter & Schlossmann (1937). Monoamine oxidase is very abundant in guinea pig liver, brain and kidney and in rat liver and brain, while it is very scarce in the rat kidney (Pugh & Quastel, 1937). It was also recently demonstrated in human placenta by Thompson & Tickner (1949). No inhibition of its activity is caused by cyanide or fluoride, although it is strongly inhibited by octyl alcohol and by 3M-urea; 0.01M-hydrazine hydrate also inhibits this enzyme.

Inhibitory action of 3M-urea and of 0.01M-hydrazine hydrate on residual oxygen uptake by the tissues during aseptic autolysis

The effect of 3M-urea and of 0.01M-hydrazine hydrate (accurately neutralized) on residual respiration of the autolysing tissue homogenates was next tested. The results, which are shown in Table 4, indicate that both those compounds in these concentrations are strongly inhibitory.

Increase of oxygen uptake on addition of tyramine and adrenaline to autolysing tissue homogenates

From the preceding experiments it appeared probable that residual O₂ uptake of the autolysing tissues was due to the activity of monoamine oxidase. Experiments were therefore carried out to study the effect of addition of tyramine and adrenaline on the residual O₂ uptake of the autolysing tissues.

The results which were obtained are presented in Table 5. Addition of tyramine strongly increases the O₂ uptake of the homogenates. Adrenaline also produces an increase, but much lower than that obtained with tyramine. This fact is in agreement with

Table 4. *Action of 3M-urea and of 0.01M-hydrazine hydrate on the residual oxygen uptake of autolysing tissue homogenates in the guinea pig*(Values expressed as $\mu\text{l. O}_2/\text{hr.}$)

Organ	Time of autolysis (days)	O_2 consumed in 1 hr. ($\mu\text{l.}$)	O_2 consumed in 1 hr. in the presence of inhibitor ($\mu\text{l.}$)	Inhibition (%)
		Urea (3M)		
Liver	20	27.5	14.1	48.7
Liver	22	32.0	11.5	64.15
Kidney	20	22.4	9.0	58.8
Liver	20	21.8	8.2	62.2
Kidney	20	10.5	2.2	78.7
		Hydrazine hydrate (0.01M)		
Liver	23	14.9	2.0	86.8
Kidney	23	9.8	4.2	56.8
Liver	20	24.4	11.2	53.8
Kidney	20	16.0	9.2	42.6

Table 5. *Oxidation of tyramine and adrenaline (0.25M) by homogenates of autolysing liver and kidney*

Animal	Time of autolysis (days)	O_2 consumed in 1 hr. ($\mu\text{l.}$)	With tyramine		With adrenaline	
			O_2 consumed in 1 hr. ($\mu\text{l.}$)	Increase	O_2 consumed in 1 hr. ($\mu\text{l.}$)	Increase
			Liver			
Guinea pig	5	80.6	208.2	+ 128.53	100.4	+ 19.75
	18	22.4	110.0	+ 87.63	38.9	+ 16.57
	18	12.8	83.2	+ 70.34	19.4	+ 6.59
	25	17.5	75.4	+ 57.86	25.3	+ 7.81
	20	25.0	42.6	+ 17.54	—	—
	21	40.1	79.2	+ 39.03	47.1	+ 6.98
Rat	15	38.8	117.9	+ 79.07	—	—
	25	37.6	52.7	+ 15.15	—	—
	25	10.6	64.9	+ 54.34	14.2	+ 3.63
	10	62.7	67.8	+ 5.12	68.2	+ 5.44
	12	42.4	—	—	51.2	+ 8.77
	15	52.0	55.6	+ 3.65	—	—
Mouse	20	12.4	25.7	+ 13.36	—	—
			Kidney			
Guinea pig	10	10.5	59.8	+ 49.32	42.6	+ 32.11
	20	21.5	38.9	+ 17.35	—	—
	25	20.7	34.7	+ 14.06	25.3	+ 4.66
	20	17.4	40.3	+ 22.91	24.1	+ 6.70
Rat	35	7.2	8.4	+ 1.18	—	—
	25	7.1	16.6	+ 9.44	9.4	+ 2.34
	25	16.5	18.6	+ 2.12	—	—
	22	3.0	12.3	+ 9.28	7.4	+ 4.38
	20	20.1	28.7	+ 8.63	22.4	+ 2.26

the findings of Pugh & Quastel (1937) and of Kohn (1937), primary amines being oxidized more readily than the secondary amines.

Increase of O_2 uptake on addition of tyramine was higher in guinea pig than in rat tissues; the values observed in the rat kidney were, in general, very low. These facts also are in agreement with the claims of Pugh & Quastel (1937), of Kohn (1937) and Schales (1947), oxidation of amines taking place very slightly in rat kidney.

The influence of O_2 tension on the oxidation of tyramine by autolysing tissue homogenates was also investigated.

The results, which are summarized in Table 6, show that 100% O_2 markedly increases the rates of oxidation of tyramine by autolysing tissue homogenates; this fact agrees with the statements of Kohn and of Thompson & Tickner (1949), who observed that monoamine oxidase activity is a function of O_2 tension.

Table 6. *Influence of oxygen tension on the oxidation of tyramine by homogenates of autolysing liver and kidney*

Animal	Organ	Time of autolysis (days)	Air			100% O ₂		
			With tyramine			With tyramine		
			O ₂ consumed in 1 hr. (μl.)	O ₂ consumed in 1 hr. (μl.)	Increase	O ₂ consumed in 1 hr. (μl.)	O ₂ consumed in 1 hr. (μl.)	Increase
Guinea pig	Liver	20	27.4	39.2	+11.77	43.7	64.7	+21.00
Guinea pig	Liver	25	25.0	42.6	+17.54	34.1	55.5	+21.38
Guinea pig	Liver	22	16.0	27.7	+11.70	26.5	48.4	+21.93
Guinea pig	Kidney	25	5.5	25.6	+20.05	21.8	52.6	+30.86
Rat	Liver	20	7.6	60.1	+52.49	14.3	93.3	+78.93
Rat	Kidney	20	5.3	18.0	+12.78	8.6	33.4	+24.83

Table 7. *Increase by addition of purified monoamine oxidase of residual respiration of tissues in aseptic autolysis*(The values were corrected for endogenous O₂ uptake of monoamine oxidase preparation.)

Animal	Organ	Time of autolysis (days)	With purified tyramine oxidase		
			O ₂ (μl./hr.)	O ₂ (μl./hr.)	Increase
Guinea pig	Liver	20	13.6	79.4	+65.39
	Liver	20	1.1	34.5	+33.46
	Kidney	20	1.2	20.8	+19.63
	Kidney	20	4.2	27.0	+22.84
Rat	Liver	25	6.7	64.9	+58.15
	Liver	25	2.7	38.9	+36.21
	Liver	26	0.9	88.1	+87.20
	Kidney	25	1.7	25.7	+23.97
	Kidney	26	1.0	27.5	+26.54
	Kidney	25	4.1	23.9	+19.79

Kohn showed that the activity of monoamine oxidase is increased 200–300 % in presence of high O₂ tensions, compared with activity in air. Thompson & Tickner were also able to confirm that the activity of the enzyme in air is only a third of the activity which occurs in presence of high O₂ tensions.

In the experiments reported above, good increases of activity were observed in 100 % O₂, but the degree increase was variable; in no experiment did the increase exceed 100 % and in a number of cases it was very much smaller. These facts suggest that, although monoamine oxidase is present in autolysing tissues, its functioning is nevertheless not optimal in these conditions.

Presence of amines in autolysing tissue homogenates

From the results of the preceding experiments the possibility arose that residual O₂ uptake by autolysing tissue homogenates was related to oxidation of amines present in these tissues. Amines could originate in the tissues by decarboxylation of amino-acids formed by catheptic hydrolysis of proteins.

Certain facts agreed with this hypothesis: (1) residual O₂ uptake is in general larger in the liver

than in kidney; (2) residual O₂ uptake is smaller in the rat kidney than in other organs; (3) monoamine oxidase activity is greater in the liver than in kidney, being particularly low in rat kidney.

These points are in agreement with the findings of Pugh & Quastel (1937), Kohn (1937) and Sloane-Stanley (1949), who observed that monoamine oxidase and amino-acid decarboxylases are generally more abundant in the liver than in the kidney and are particularly scarce in rat kidney.

To verify this hypothesis, experiments were carried out to demonstrate the presence of free amines in autolysing tissue homogenates.

For these purposes an indirect criterion was employed, consisting of the measurement of extra O₂ uptake by the tissue homogenates when a purified monoamine oxidase preparation was added. O₂ uptakes, larger than the theoretical amounts (which result from the sum of endogenous O₂ uptake of the tissue homogenate plus the amount of O₂ consumed by the purified enzyme preparation), show the presence in the tissue homogenate of a substrate for added monoamine oxidase. Since the specificity of purified monoamine oxidase for amines has been demonstrated by Kohn (1937), it seemed likely that

Table 8. *Consumption of oxygen and production of ammonia by autolysing tissue homogenates in absence and presence of tyramine*

Animal	Organ	Time of autolysis (days)	Tyramine absent		Tyramine present		Atoms O ₂ : mol. NH ₃	
			O ₂ consumed in 1 hr. (μl.)	NH ₃ (μmol./hr.)	O ₂ consumed in 1 hr. (μl.)	NH ₃ (μmol./hr.)	Tyramine absent	Tyramine present
Guinea pig	Liver	20	25.1	1.08	42.7	2.06	2.06	1.83
	Liver	20	17.0	0.72	29.4	1.58	2.09	1.65
	Liver	22	27.3	1.32	59.5	3.62	1.83	1.43
	Kidney	20	21.5	0.98	38.9	2.74	1.94	1.25
Rat	Liver	25	17.7	0.80	40.1	2.42	1.96	1.47
	Kidney	25	6.4	0.30	12.0	0.82	1.88	1.30

a positive proof could be a valid argument for the presence of free amines in the autolysing tissue homogenates. The results of these experiments are reported in Table 7, and it is concluded that they provide evidence for the presence in autolysing tissue homogenates of a substrate for monoamine oxidase.

Consequently, it seems likely that the small amounts of O₂ which are taken up by these homogenates, even many days after the beginning of

amines. The quotient is lower and nearer to the theoretical values in the experiments with tyramine.

(b) *Production of hydrogen peroxide* during residual respiration of the autolysing tissues was also studied. The results of experiments based on the observations of Keilin & Hartree (1936), involving the addition of 1 mg. of ethanol to the homogenates, show that ethanol is very slightly oxidized. One such experiment is summarized below:

Animal	Organ	Time of autolysis (days)	O ₂ (μl./hr.)	O ₂ (μl./hr.) in presence of 1 mg. ethanol	Increase
Rat	Liver	20	19.5	23.1	+ 3.61

autolysis, are due to oxidation of amines present in the tissues by the surviving specific enzyme.

Presence of products of amine oxidation in autolysing tissue homogenates

Further studies were performed to show the possibility of occurrence in the autolysing tissues of products of oxidation of amines. According to Kohn, as a consequence of oxidation of amines, an aldehyde, hydrogen peroxide and ammonia are produced.

(a) *Production of ammonia* was first ascertained qualitatively with Nessler's reagent; afterwards, quantitative determinations were performed. Results are given in Table 8, from which it may be seen that ammonia is produced during residual respiration of autolysing tissues; NH₃ production increases remarkably in the presence of tyramine. In further experiments it was shown that an environment of 100% O₂ can noticeably increase both O₂ uptake and ammonia production.

The calculation of the quotient 'atoms O₂ consumed: mol. NH₃ produced' shows that, in the absence of tyramine, the quantity of O₂ which is consumed is approximately double the theoretical amount: this fact could mean that only one half of O₂ uptake is related to the actual oxidation of free

Since small extra O₂ uptakes observed in the presence of ethanol cannot be referred to the activity of ethanol dehydrogenase (on account of the inactivity of cytochrome oxidase in the autolysing tissues and of the presence of cyanide in the reaction mixture), it is likely that the phenomenon is a consequence of the oxidation of added ethanol by H₂O₂ which is produced in traces in the homogenates.

Also the second indirect criterion which was employed for the demonstration of production of H₂O₂ during residual respiration of autolysing tissues (oxidation of Hb to meta-Hb) gave positive results. At the end of the experimental period obvious changes had occurred in the colours of the fluids contained in the flasks; while the control solution remained a rose-red colour, the colour of the solution containing the filtrate of the tissue homogenate + Hb was brown.

(c) *Presence of aldehydes*. Extra O₂ uptake after addition to the homogenates of a purified xanthine oxidase (Schardinger's enzyme) was considered as proof of the presence of aldehydes in the homogenates.

Results of two experiments are reported below. (The values of O₂ uptake in the presence of the xanthine oxidase preparation were corrected for the endogenous O₂ uptake of the enzyme.)

Animal	Organ	Time of autolysis (days)	O ₂ (μl./hr.)	O ₂ (μl./hr.) in presence of xanthine oxidase preparation	Increase
Guinea pig	Liver	24	18.7	23.1	+ 4.38
	Kidney	24	13.3	18.5	+ 5.2

From these experiments it seems that addition of Schardinger's enzyme causes a small increase of O_2 uptake.

Additional experiments showed also that, when xanthine oxidase is added to the homogenates after 1 hr. of residual respiration rather than at the start of the experiment, O_2 uptake during the next half-hour shows slightly larger increases. The explanation of this may be that aldehydes are not pre-existing in the homogenates, but are produced during the period of respiration.

DISCUSSION

It is concluded from the experiments reported in this paper that a considerable amount of the residual O_2 uptake by homogenates of autolysing tissues is related to the oxidation by surviving monoamine oxidase of free amines which are present in these tissues.

Amines may be produced in autolysing tissues by catheptic hydrolysis of proteins and by decarboxylation of the amino-acids so produced. Catheptic hydrolysis of proteins can occur in the given experimental conditions (pH 4.5, absence of O_2); amino-acid decarboxylases are also active anaerobically. Monoamine oxidase, which shows optimal activity in weakly alkaline media and is dependent for its functioning upon the presence of O_2 , cannot act, on the contrary, in the experimental conditions used. Accumulation of free amines could consequently occur.

This interpretation is in agreement with Kohn's finding, which was also confirmed in the present work, that in purified monoamine oxidase preparations kept in the cold store, endogenous O_2 uptake increases remarkably in the course of time, a likely consequence of liberation of substrate.

Monoamine oxidase itself undergoes some destruction in tissues which have been exposed for 20–30 days to autolysis, residual O_2 uptake and intensity of oxidation of tyramine decreasing after the first few days of autolysis; it is completely destroyed after 30–40 days.

From the experiments on ammonia production, it is concluded that the quotient 'atoms O_2 consumed:

mol. NH_3 produced' is always greater than the theoretical, and it seems likely therefore that other oxidative processes, perhaps of minor importance, can occur during the residual respiration of the autolysing tissue homogenates.

Since conditions very similar to those used experimentally in the present work occur in ischemic necrosis of tissues, it seems possible that similar chemical processes may occur in ischemic necrotic tissues. This possibility may be of particular interest in connexion with the aetiology of certain hypertensive states.

SUMMARY

1. It has been confirmed that liver and kidney of guinea pigs, rats and mice undergoing aseptic autolysis can consume small quantities of oxygen even many days after the beginning of autolysis.

2. pH optimum for residual respiration of autolysing tissues occurs in a weak alkaline medium (pH 7.4–7.6); oxygen uptake increases noticeably in the presence of high oxygen tensions; cyanide and fluoride exert only a weak inhibitory action on the residual respiration, while *sec.*-octyl alcohol, 3M-urea and 0.01M-hydrazine hydrate inhibit strongly.

3. Succinoxidase, lactic dehydrogenase, α -glycerophosphate dehydrogenase, and D- and L-amino-acid oxidase are completely inactive in autolysing tissues.

4. Addition of tyramine or adrenaline hydrochlorides produces a marked increase of oxygen uptake.

5. Addition of purified monoamine oxidase to homogenates of autolysing tissues produces an increase of oxygen consumption, showing the presence of free amines.

6. Evidence is adduced for the production of ammonia and of traces of hydrogen peroxide and aldehyde compounds during residual respiration.

7. The hypothesis is presented that residual respiration of autolysing tissue homogenates is related to the oxidation of free amines present in the homogenates by a surviving monoamine oxidase.

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