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Studies of Drugs in Tissue Metabolism. II. Action of Drugs on Metabolism of Tissue in Serum.

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The present paper is a study of the action of certain drugs on surviving tissue cells, respiring in serum. Specifically, it deals with the effect of the hypnotics, chloral hydrate and barbital; and of certain dyes, particularly methylene blue, on the respiration of liver tissue in human serum. The experimental technic employed in this study has been described in the preceding paper. All experiments were performed in duplicate or triplicate.

Methylene Blue. Barron has shown that this dye stimulates the oxygen consumption of red blood cells and of cells poisoned with KCN,[1, 2] but does not increase the oxygen consumption of normal liver or kidney tissue suspended in phosphate-Ringer solution.[2] The effect of this dye on the oxygen consumption of liver when added to serum was, therefore studied in order to determine whether liver in serum might not carry out oxidative reactions not possible in a simple salt solution. Using human serum, we found, to our surprise, that there was an increase of approximately 50% in oxygen uptake upon adding the dye in a concentration of 0.5 mg/cc, while dye concentrations of 0.05 mg/cc and 5.0 mg/cc gave an increase of approximately 20%, as shown in Table I.

On the other hand, in confirmation of Barron,[2] we found that methylene blue added to phosphate-Ringer's caused no increase in the oxygen consumption of rat liver. Nor did the addition of 4 other dyes (Congo red, rose Bengal-blue, brilliant vital red and Evans blue, all of which are sometimes used in clinical work) affect the

TABLE I.
Effect of Adding Methylene Blue to Serum on Oxygen Consumption of Rat Liver.

Rat No.	Conc. dye mg/cc	Avg Q_{O_2} without dye	Avg Q_{O_2} with dye	% increase
1	.05	—11.5	—14.1	22.6
2	.5	—12.3	—18.3	48.8
3	.5	—11.0	—16.9	53.6
4	.5	—12.4	—17.5	41.0
5	.5	—11.4	—16.9	48.2
6	.5	—14.6	—21.8	49.3
7	5.0	—12.1	—14.3	18.2

1 Barron, E. S. G., and Harrop, G. A., Jr., *J. Biol. Chem.*, 1928, **79**, 65.

2 Barron, E. S. G., *J. Exp. Med.*, 1930, **52**, 447.

metabolism of liver suspended in serum. It is concluded from these observations that the reversibly oxidizable dye, methylene blue has the ability in the presence of serum of increasing the oxygen consumed by rat liver slices *in vitro*. What portion of the oxidation system is affected is not known. It is interesting to note that in serum a large dose of the dye (5 mg/cc) does not give as much oxygen consumption as the smaller dose of 0.5 mg/cc, thus indicating that such large doses may be toxic to the tissue.

Chloral Hydrate. Chloral hydrate has been shown by Jowett[3] to exert a depressing effect on the oxygen consumption of brain cells, in doses ranging from 0.15-0.9 mg/cc using phosphate-Ringer's media. We have studied the effect of toxic concentrations of chloral hydrate in serum, (3.3 mg/cc) on the oxygen consumption of liver. As shown in Table II, there was a marked decrease, approximately 68%, in the oxygen consumption of liver tissue which is in agreement with comparable observations made by Jowett and Quastel,[4] on the action of such hypnotics.

Chloral Hydrate and Methylene Blue. In view of the observation by Barron[2] that certain reversible dyes will increase the metabolism of cells after inhibition by certain poisons, and of our observation that methylene blue stimulates oxygen consumption of liver tissue in serum media, this dye was added to serum containing chloral hydrate to determine whether it exerted any effect on the oxygen consumption of tissue after its depression by the narcotic. These results are shown in Table III.

TABLE II.
Action of Chloral Hydrate (3.3 mg per cc) on Rat Liver Suspended in Human Serum.

Rat No.	Q_{O_2} without chloral	Q_{O_2} with chloral	% depression of Q_{O_2}
1	—12.5	—4.3	65
2	—12.5	—4.1	67
3	—13.5	—4.6	66
4	—14.6	—3.4	77

TABLE III.
Action of Methylene Blue in Concentration of 0.5 mg per cc on Rat Liver Poisoned with Chloral Hydrate.

Rat No.	Conc. of chloral hydrate mg/cc	Q_{O_2} without dye	Q_{O_2} with dye	% increase in Q_{O_2}
1	2.0	—3.7	— 5.3	43.3
2	3.3	—4.6	— 8.4	82.6
3	3.3	—6.0	—14.0	133.0
4	3.3	—4.3	— 9.3	116.0

[3] Jowett, M., *J. Physiol.*, 1938, **92**, 322.

[4] Jowett, M., and Quastel, J. H., *Biochem. J.*, 1937, **31**, 565.

It was found, as this table shows, that the dye greatly increased the oxygen consumption after inhibition by chloral hydrate, in some experiments restoring the Q_{O_2} to an approximately normal value. Whether or not this means that the normal oxidation processes of the cells were restored cannot be stated, although it is probable that the chloral inhibited a necessary, reversibly oxidizable component which was replaced by the dye.

Sodium Barbital (sodium di-ethyl-barbiturate) and Methylene Blue. In view of the observations of Jowett and Quastel[4] that luminal (phenobarbital) and evipan (sodium N-methylcyclohexenyl-methylbarbiturate) depress the oxygen consumption of brain and liver in phosphate-Ringer's, a study was made of the action of one drug of this series (sodium barbital) in serum media. The results are presented in Table IV. It was found that barbital in a concentration of 3.3 mg per cc depressed the metabolism of liver in serum from 13 to 34%. In contradistinction to the stimulating effect of methylene blue on the metabolism of liver after chloral hydrate, the dye had no such effect on the barbital depressed tissue. Indeed, there was a further decrease in oxygen consumption. This is to be interpreted as indicating that the barbital decreased the oxidative processes of the tissue cells by inhibiting quite a different portion of the oxidation system than that affected by the chloral.

Comment. The observations reported above indicate that the method of measuring the metabolism of rat liver in human serum is suitable for the study of the action of drugs on the oxidative system of tissue cells. It has also been shown by the opposite effects produced by a reversibly oxidizable dye, such as methylene blue, on the metabolism of liver depressed by chloral and by barbital, that a difference in their mode of action is indicated. It is, perhaps, of interest that in 3 comparable experiments with morphine added to serum in rather large amounts (1 mg per cc), no effect on the respiration of liver was found. So far, no study has been made of serum from patients who had received the drugs studied in the present paper.

TABLE IV.
Action of Sodium Barbital (Concentration 3.3 mg per cc) and Methylene Blue (Concentration .5 mg per cc) on Oxygen Consumption of Liver in Serum.

Exp. No.	Q_{O_2} with barbital cmm/mg/hr	% depression*	Q_{O_2} with barbital and dye cmm/mg/hr	% depression*
1	10.9	13	10.2	18
2	9.9	21	7.0	44
3	8.2	34	6.4	49

*Standard normal liver $Q_{O_2} = 12.5$.

However, it has been shown by Friend and Robinson,[5] using this technic, that sodium thiocyanate exerts a depressant action on liver metabolism both when added to serum *in vitro* and when present in blood serum as drawn. A hitherto unpublished experiment, furthermore, showed that serum from a patient to whom a large amount of thiocyanate had been administered depressed the liver metabolism 30%. The addition of methylene blue to this serum restored the liver metabolism to its normal Q_{O_2} of 13.0.

Summary. The effect on liver metabolism of adding methylene blue, chloral hydrate and sodium barbital to serum has been studied. Methylene blue has been found to increase the oxygen consumption of rat liver respiring in human serum. Chloral hydrate and sodium barbital both depress the metabolism. Following depression of the oxygen consumption by chloral hydrate, methylene blue increases the metabolism. Following sodium barbital, the dye further depresses the metabolism. This is interpreted as indicating a difference in the site of action of the drugs on the oxidative system of the tissue cells.

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Excretion and Determination of Cinchophen in Bile.

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It has been established that cinchophen has a choleretic action in man and dog.[1-7] It does not apparently stimulate bile volume output in the rabbit.[8] The effect of cinchophen on the output of the various constituents of bile is disputed in the literature,[1-7] and good quantitative data on the excretion of cinchophen in the bile could not be found by us. However, cinchophen is known to be excreted in bile;[2]

5 Friend, D. G., and Robinson, R. W., *J. Lab. Clin. Med.*, 1939, **24**, 832.

1 Brugsch and Horsters, *Z. ges. exp. Med.*, 1923, **38**, 367.

2 Taubmann, *Arch. exp. Path. u. Pharm.*, 1927, **121**, 204.

3 Chabrol and Maximin, *Press. Med.*, 1929, **37**, 666.

4 Taschenberg and Hofmann, *Deut. med. Wochn.*, 1925, **51**, 1611.

5 Speerling and Hartman, *J. Lab. and Clin. Med.*, 1928, **13**, 854.

6 Horsters, *Arch. exp. Path. u. Pharm.*, 1925, **105**, xi.

7 Goffin, *Compt. rend. Soc. de Biol.*, 1936, **123**, 97.

8 Strausky, *Biochem. Z.*, 1926, **155**, 256.