in progress, it is assumed that benzene and phenanthrene are conjugated in the animal body to yield, perhaps, a mercapturic acid type product, similar to p-halogen-phenylmercapturic and l-α-naphthalene mercapturic acids.

8295 C

Effects of Various Anesthesias on Autoxidation Rate of Surviving Brain Tissue.

GEORGE A. EMERSON. (Introduced by Paul D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.

It has previously been shown that ether anesthesia in rats brings about a condition, demonstrable in brain tissue removed immediately after termination of one hour of deep surgical anesthesia, such that the rate of autoxidation of available carbohydrate and lactic acid in the surviving brain decreases more rapidly than is usual for tissues taken from untreated rats.[1] Since both the total carbohydrate and glycogen content of rat brain decreases in ether anesthesia[2] despite the marked hyperglycemia maintained at the same time, it was suggested that the inhibitory effect on rate of autoxidation was due simply to limiting of oxidizable carbohydrate in the excised tissue. The ability of such tissue to metabolize added glucose at a normal rate substantiates this.

Examination of data on blood and urine chemistry shows marked resemblance of biochemical effects of epinephrine and ether. The possibility that many of the physiological side actions of ether are mediated through stimulation of adrenin output is supported by the weight of much evidence,[3] and studies on intestinal chemistry[4] and ether ketosis[5] show marked parallelism of many effects of the 2 agents. Unequivocal direct evidence of the supposed action of ether on the suprarenals is as yet lacking.[6] The present communica-

[1] Emerson, G. A., *J. Tenn. Acad. Sci.* In press.

[2] Uchida, S., *Biochem. Z.*, 1926, **167**, 9.

[3] Knoefel, P. K., *California and Western Med.*, 1933, **39**, 5.

[4] Emerson, G. A. To be published.

[5] Emerson, G. A., *J. Pharm. Exp. Therap.*, 1935, **54**, 90.

[6] Elliott, T. R., *J. Physiol.*, 1912, **44**, 374; Schlossmann, H., and Mügge, H., *Arch. Exp. Pathol. Pharmacol.*, 1929, **144**, 133; Fujii, I., *Tohoku J. Exp. Med.*, 1925, **5**, 566; Kodama, S., *Tohoku J. Exp. Med.*, 1924, **4**, 601.

tion is concerned with demonstrating whether other narcotics having some sympathomimetic actions in the organism effect changes in rate of autoxidation of surviving brain tissues taken from anesthetized rats.

Five to 10 individual experiments were carried out with each agent tested, and single typical responses selected for Table I. The direct method[7] of measuring respiration in a Warburg apparatus was used. Consequently there was lacking the same check on the disturbing influence of possible variations in the partial pressures of foreign anesthetic gases as was easily obtained[1] in the indirect method of Warburg by examining the magnitude of CO_2 production in excess of theory. However, since the present results agree well for the case of ether with those previously obtained by the indirect method, it may be assumed for the present purposes that the results with other agents are likewise real and not artefacts due to either liberation or absorption of the anesthetic gas by the fatty brain tissue during the course of measurement of respiration.

TABLE I.
Oxygen Consumption of Surviving Brain Tissue from Anesthetized Rats During Autoxidation.

Anesthetic Agent	$-Q_{O_2}$* Time of Autoxidation, Min. 15	30	45	60	75	After Glucose, 0.2% 30
Ether	9.4	4.6	3.8	2.6	2.2	8.2
Divinyl Oxide	7.4	7.2	5.0	4.2	3.6	5.8
Ethylene	6.8	6.76	5.64	4.6	4.4	6.7
Cyclopropane	7.0	5.2	4.4	4.0	3.7	5.06
Nitrous Oxide	8.2	7.2	6.24	4.16	3.6	8.0
Ethanol	10.6	8.4	5.8	5.0	4.8	8.2
Dihydromorphinone	7.1	5.7	4.9	4.6	4.4	6.3
Chloroform	8.4	5.1	4.7	3.3	3.1	7.4
Epinephrine	7.4	5.2	4.6	3.6	2.4	7.2
Control	8.2	7.1	6.4	5.8	5.2	7.8

*$-Q_{O_2}$ expressed as mm.^{3} of O_2 absorbed per hour per mg. dry weight of tissue; calculated for each 15-minute interval terminating at the time above.

Rats were anesthetized for one hour by placing them in an 18 l. chamber initially containing 2.5 mM/l. of ether, 2.0 mM/l. of divinyl oxide or 0.8 mM/l. of chloroform in oxygen, the mixtures being made up by Fühner's method.[8] For the anesthetic gases, mixtures with oxygen were made up by using a flow-meter apparatus, and were passed through a 10 l. chamber in which the rats were placed for 1 hour. The following concentrations were employed:

[7] Dixon, M., and Elliott, K. A. C., *Biochem. J.*, 1930, **24**, 820.

[8] Fühner, H., *Biochem. Z.*, 1921, **115**, 235.

ethylene, 85%; cyclopropane, 20%; and nitrous oxide, 90% in oxygen. Five ml./kg. of ethyl alcohol, in a 40% aqueous solution, were injected intraperitoneally in 5 rats and 0.5 mg./kg. of epinephrine hydrochloride or 5.0 mg./kg. of dihydromorphinone hydrochloride (Dilaudid, N. N. R.) subcutaneously in 10 rats.

The whole brain of each anesthetized rat was quickly excised, minced and a 50 to 100 mg. aliquot weighed to the nearest 0.5 mg. at the end of an hour after beginning administration of the various agents. The weighed tissue was quantitatively transferred to a Warburg manometer vessel containing 3.0 ml. of a balanced physiological salt solution at pH 7.4 and equilibrated with oxygen for 10 minutes at 37° before commencement of measuring respiration. The entire process from the time of excising the brain tissue to beginning respiration measurements thus occupied about 15 minutes. At the end of one and a quarter hours of autoxidation, glucose was tipped from a side pocket in the manometer vessel into the menstruum about the tissue to make a final concentration of 0.2%, and respiration followed for another 30 minutes. Control tissues from the brains of untreated rats were included in each experimental run, and duplicate aliquots taken as checks in about half the cases. The pH of the contents of the manometer vessels was checked in all cases at the end of the experiment.

Quastel and Wheatley[9] have shown that while the initial autoxidative rate of brain tissues from unselected animals of any species may vary over a wide range, comparable results on different brains may be obtained if the tissues are exhausted by autoxidation until the reserves of glucose and lactate are depleted, whereupon addition of available substrate produces fairly uniform responses. This implies that the activity of the dehydrogenase systems in normal brain tissue is also reasonably uniform, and that the initial autoxidation rate is dependent on the amount of available carbohydrate present at the moment, while the decrease in autoxidation rate is a measure of the amount of reserve carbohydrate. Thus in the present work it was found that the initial autoxidative rate was not significant and may vary from 6.5 to 10.5 mm^3. of O_2 per hour per mg. of dry weight of tissue, while the rate of decline of autoxidation is sufficiently constant in different brain tissues taken from untreated fed rats to permit significant comparison with experimentally treated animals. Such results are included in Table I. In order to magnify the decrease in autoxidative rate, oxygen consumptions expressed as $-Q_{O_2}$ over single consecutive 15-minute intervals are given rather

[9] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 1932, **26**, 725.

than the summated $-Q_{O_2}$ for the entire time period, as is customary.

The present results should not be taken to indicate direct effects of the narcotics studied upon oxidative processes in the brain. As has been pointed out by Quastel and Wheatley,[10] such studies should be done with tissue taken from narcotized animals which is subsequently allowed to oxidize in media containing approximately that concentration of the narcotic agent which may be found in sera of deeply narcotized animals. Even with agents highly soluble in lipoids, the concentration is effectively reduced by placing the tissue in 30 to 60 volumes of aqueous media, as is done in the present experiments. The positive effects here noted are better interpreted as being due to biochemical changes in the tissue not directly related to the presence of low concentrations of narcotics in the brain; the one exception to this may be in the case of ethanol.

Ether and chloroform anesthesias and massive injection of epinephrine hydrochloride were found to inhibit the autoxidative rate significantly (Table I). If results in all the experiments are to be interpreted as modified by sympathetic activity, the slight effects with nitrous oxide might be referred to that dependent on the slight anoxemia present; with divinyl oxide, to a slight direct sympathetic effect or through anoxemia brought about by the primary increased flow of mucus and resultant faulty gas exchange; and with cyclopropane to a relatively small direct effect on the sympathetic nervous system.

Results with ethanol confirm those of Robertson and Stewart[11] and Wortis,[12] although the extra uptake of O_2 cannot be considered very great. Dilaudid has apparently less activity on the autoxidative rate than might be expected from its hyperglycemic action; with morphine, Gross and Pierce[13] have reported marked increases in autoxidative rates of brain tissue from non-tolerant rats receiving 50 mg. of morphine sulphate per 100 gm., above the lethal dose. In view of the decrease in total carbohydrate content of the brain after morphine, reported by Uchida,[2] this suggests that other biochemical mechanisms than the simple limiting of substrate or enzyme system are involved. The findings of increased autoxidative rate[12] in tissues from rats receiving massive doses of glucose + a small dose of epinephrine insufficient to interfere with carbohydrate metabolism, do not contradict the present results showing a decrease after admin-

10 Quastel, J. H., and Wheatley, A. H. M., *Proc. Roy. Soc., B*, 1932, **112**, 60.

11 Robertson, J. D., and Stewart, C. P., *Biochem. J.*, 1932, **26**, 65.

12 Wortis, S. B., *Arch. Neurol. Psychiat.*, 1935, **33**, 1022.

13 Gross, E. G., and Pierce, I. H., *J. Pharm. Exp. Therap.*, 1935, **53**, 156.

istration of a massive dose of epinephrine without glucose. Especially applicable to the explanation of the present findings are the data of Wortis[12] illustrating marked depression of autoxidation in rats fasted 2 to 3 days and given insulin.

The inconstancy of the normal glycogen content of brain tissue of rabbits found by Holmes and Holmes[14] doubtless has some influence on the constancy of control rates of brain autoxidation. The data of Uchida[2] show regular decreases of glycogen and total carbohydrate content of rat brain after administration of various narcotics, however, and these are reflected in results on autoxidation rates if a sufficiently large series of rats are used. Variations in the concentrations of lactic acid or other oxidizable substrates are not considered here.

Table I illustrates that the effect of anesthesia with various narcotics on the rate of autoxidation of surviving brain differs markedly with the chemical nature of the narcotic, as the depth of anesthesia was designed to be approximately equivalent for all cases. It is known that the agents studied differ markedly in their glycogenolytic potencies, and on the assumption that some correlation might be obtained between this and the autoxidative rate changes, the effect of these agents on blood sugar during narcosis was studied. As the brain respiration studies were carried out on fed male rats, glucose determinations were similarly done on fed rats from the same source, though less consistent results were so obtained than if the rats had been fasted as in previous work on glucose tolerance in normal and leprous rats.[15] After 2 control samples of blood had been drawn intracardially from rats at 10-minute intervals, the rats were treated with the same concentrations of anesthetic agents as previously for one hour. Heart blood was obtained at 10-minute intervals from each rat during the time of anesthesia and the glucose content estimated by the Hagedorn-Jensen[16] micro-method. The results of some 260 determinations are summarized in Table II.

As in other species, ether and chloroform anesthesias and epinephrine bring about a marked hyperglycemia in rats, cyclopropane and nitrous oxide a smaller rise in blood sugar, and ethylene and the necessary experimental technique none. Divinyl oxide anesthesia apparently causes a very sudden increase which is not sustained; this may be due to both a slight direct effect and an anoxemia

14 Holmes, B. E., and Holmes, E. G., *Biochem. J.*, 1925, **19**, 492, 836; 1926, **20**, 1196.

15 Emerson, G. A. To be published.

16 Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46; **137**, 92.

TABLE II.
Comparative Action of Anesthetics on Blood Sugar of Fed Rats.
Results Expressed as mg. % Glucose.

Treatment	No. Rats	Preanesthetic Controls		Time of Anesthesia, Min. 10	20	30	40	50	60
Diethyl ether, 2.5 mM/l.	6	124	124	167	203	212	224	226	235
Divinyl oxide, 2.0 mM/l.	5	109	113	198	183	163	153	150	132
Ethylene, 85% in O_2	3	111	109	115	109	109	111	114	114
Cyclopropane, 20% in O_2	4	114	113	145	127	123	145	141	130
Nitrous oxide, 90% in O_2	3	124	118	137	151	149	172	159	169
Chloroform, 0.8 mM/l.	5	121	124	145	162	182	192	191	197
Epinephrine Hydrochloride 0.5 mg./Kg., subc.	5	126	124	173	216	233	270	282	279
Untreated Controls	2	115	108	107	112	116	111	109	112

effected by the marked salivation which occurs only during induction with this agent. The absence of a sustained marked hyperglycemia in deep surgical anesthesia with divinyl oxide confirms experimental work of Phatak[17] on larger mammals. With the small series of rats studied here, the standard deviations of the groups are high; this value varies in the different groups, for the glucose determinations taken after 30 minutes of anesthesia, from ±2 mg. % for ethylene to ±13 mg. % for ether and ±14 mg. % for chloroform.

Isoamylethylbarbituric acid (Amytal, N. N. R.) may inhibit glycogenolysis normally occurring in ether anesthesia[18] in which no preanesthetic medication has been given, as it does in the case of other glycogenolytic narcotics.[19] Amytal was found to inhibit the effect of ether anesthesia on autoxidation of surviving brain tissue as well. (Table III.) Three rats were anesthetized with 2.0 mM/l. of

TABLE III.
Single Experiment Illustrating the Effect of Amytal Premedication on Ether Inhibition of Autoxidation in Surviving Brain.

Treatment	$-Q_{O_2}$ Time of Autoxidation, Min. 15	30	45	60	75	After Glucose, 0.2% 30
Ether, 2.0 mM/l.	7.40	5.70	4.20	3.50	3.10	6.70
Ether + Amytal	6.40	5.86	5.60	5.24	4.90	6.00
Untreated	7.60	6.40	6.10	6.00	5.80	6.80

ether for 30 minutes; of these, 2 had been given 20 mg./kg. of amytal 15 minutes before induction of anesthesia. Since amytal alone has no significant action on autoxidation,[12] the results demon-

17 Phatak, N. M., unpublished data.

18 Knoefel, P. K., *J. Indiana State Medical Assn.*, 1935, **28**, 217.

19 Olmsted, J. M. D., and Giragossintz, G., *J. Lab. Clin. Med.*, 1931, **16**, 354.

strate the dependence of the autoxidative rate upon glycogenolysis during anesthesia.

Summary. The present findings of decreased autoxidative rates in brains of rats anesthetized with glycogenolytic narcotics or treated with epinephrine are in accordance with the concept of general mediation of many biochemical processes during anesthesia through stimulation of adrenin output, with the possible exception of Dilaudid. In other cases studied, blood sugar curves show an inverse relationship to the rate of autoxidation after administration of narcotics, and no direct correlation with the depth of anesthesia produced by different anesthetics.

8296 C

Influence of Acid Extract of Cattle Anterior Pituitary Gland on Bone Repair in Young Guinea Pigs.

MARTIN SILBERBERG AND RUTH SILBERBERG. (Introduced by Leo Loeb.)

From the Department of Pathology, Dalhousie University, Halifax, N. S.

We have shown[1] that a growth-promoting effect is exerted by acid extract of cattle anterior pituitary gland on bone and cartilage in young guinea pigs. The present investigation deals with the influence of this extract on callus formation and healing of fractured bones.

Twenty-four fall and winter guinea pigs averaging from 130 to 300 gm. in weight were used in these experiments. Under deep ether anesthesia both tibia and fibula of the left hind leg as a rule were fractured in these animals. As exposure of the bone with the unavoidable removal of the periosteum was followed by unsatisfactory healing, the following procedure was used: After shaving the skin of the leg the bones were broken at about the middle of their shafts. The broken ends were carefully approximated, the leg fixed with a small wooden splint and tightly bandaged with adhesive plaster. Sixteen animals thus operated on were injected with from 1 to 1½ cc. of extract daily, while 8 others of similar size, weight and age were not injected and kept under the same environmental factors, thus serving as controls. All were fed in the same manner with oats and bran or shorts. In addition, they received some cabbage, hay, lettuce and turnips. The injections were continued for periods

[1] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423.