genases and cytochrome *c* oxidase) are little affected by *in vivo* irradiation at lethal levels.

Summary. Rats were subjected to 700 r of whole-body X-irradiation. At intervals of 13 to 85 hours after exposure, homogenates were prepared from spleens of irradiated and control animals and separated into 4 subcellular fractions by differential centrifugation. Per mg of nitrogen, DPNH cytochrome *c* reductase specific activity of the homogenates from X-rayed rats was increased significantly over the control levels at 13 and 37 hours after exposure. At 85 hours, the difference was much less owing to an increased specific activity of the enzyme in the control homogenates, as well. Expressed on the basis of nitrogen, cytochrome reductase specific activities of nuclear, microsomal, and supernatant fractions after irradiation showed no striking changes as compared to control values. Specific activities of the mitochondrial fractions from spleens of irradiated animals were decreased significantly as compared to control levels. The % of total DPNH cytochrome *c* reductase activity recovered in the 4 fractions prepared from the spleens of irradiated and control animals was essentially the same.

1. Maxwell, E., and Ashwell, G., *Arch. Biochem. and Biophys.*, 1953, v43, 389.

2. Van Bekkum, D. W., Jongepier, H. J., Nieuwerkerk, H. T. M., and Cohen, J. A., *Trans. Faraday Soc.*, 1953, v49, 329.

3. Van Bekkum, D. W., in *Radiobiology Symposium*, (Bacq, Z. M., and Alexander, P.) p201, Butterworths Scientific Publications, London, 1955.

4. Potter, R. L., as quoted by Ord, M. G., and Stocken, L. A., *Brit. J. Radiol.*, 1955, v28, 279.

5. Hogeboom, G. H., *J. Biol. Chem.*, 1949, v177, 847.

6. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.*, 1948, v172, 619.

7. Schneider, W. C., and Hogeboom, G. H., *J. Nat. Cancer Inst.*, 1950, v10, 969.

8. Potter, V. R., and Albaum, H. G., *J. Gen. Physiol.*, 1943, v26, 443.

9. Gutcho, S., and Stewart, E. D., *Anal. Chem.*, 1948, v20, 185.

10. Racker, E., *J. Biol. Chem.*, 1950, v184, 313.

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Effects of Adrenergic and Cholinergic Drugs Injected by Intra-Carotid Route on Electrical Activity of Brain. (23112)

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It has previously been reported(Longo, 10) that the EEG pattern provoked by intracarotid injection of acetylcholine (ACh) in rabbits is similar to the "activation" or "arousal" response produced by external stimuli. Similar observations have been made by Rinaldi and Himwich(15), who consider this effect as a specific action of cholinergic drugs on the reticular activating system. On the other hand, much experimental data suggest that cholinergic drugs are not the sole mediators of the activation response. In fact, it has been demonstrated that epinephrine and amphetamine are also able to elicit an activation picture in the EEG of experimental animals(Bonvallet *et al.*, 1; Elkes *et al.*, 6; Schallek and Walz, 17). This paper is concerned with the modifications of EEG after injection of acetylcholine, eserine, epinephrine, and amphetamine directly into the brain circulation.

Methods. Fourteen non-curarized unanesthetized rabbits and 12 "cerveau isolé" preparations (for details of "cerveau isolé" preparation in rabbit, *cf.* Longo and Silvestrini, 12) were used. Blood pressure was measured in majority of experiments, connecting femoral artery to mercury manometer. Some additional experiments were carried out on cats curarized with d-tubocurarine. The technic(Longo, 10) consists in cannulating the internal carotid in the rabbit so that the injected drug reaches the brain directly. Precautions were taken to avoid arousal reactions

caused by external solicitation during the injection, and repeated controls were made injecting saline. Cortical and subcortical EEG were recorded by technics reported elsewhere (Longo, 11). Eserine and amphetamine were injected in the form of salicylate and sulfate salts respectively; acetylcholine, either as hydrochloride or perchlorate salt, epinephrine in the form of bitartrate. All doses are reported as total amount given to animal and are expressed as weight of free bases. At the end of the experiment, an intracarotid injection of methylene blue or of India ink was given for macroscopic control of the area that this route supplies.

Results. Administration of drugs into internal carotid of normal rabbit. Acetylcholine. In all of the animals an arousal response was first elicited by injecting intracarotid ACh. The results obtained previously (Longo, 10) were confirmed. All animals responded to ACh; however, effective doses varied in different animals from 0.5 to 2 μg. The injection produced a desynchronisation of the tracing with very short (1-2 seconds) latent periods. This EEG arousal was generally accompanied by tachypnoea and searching movements of the head, with higher doses (10-20 μg) by efforts to escape. Repeated administration of ACh given at intervals of 10 minutes provoked always the same response in the EEG. The records of the blood pressure showed that no modification followed the small doses; a delayed and short lasting fall was noticed after high doses. The same high dose of ACh, by intravenous injection, produced considerable lowering of blood pressure and flattening of the EEG, but no arousal reaction.

Eserine. The general procedure followed was to administer only one drug in every experiment after sensitivity to ACh was tested. Five animals received eserine. A single dose of 10 μg was never followed by EEG changes. When a total amount of 30 to 50 μg was reached by repeated administration or in a single administration, a distinct activation of the tracing appeared after a latent period of 1-2 minutes and lasted for 30 minutes or more. Attention should be drawn to the fact that external stimulation of the animal immediately after injection of the drug reduces the latent period, as it gives rise to prompt and prolonged desynchronisation. These EEG patterns of arousal were not accompanied by behavioural changes seen after injection of ACh.

Epinephrine. In 4 rabbits injection of doses up to 20 μg caused no modifications of the EEG. When the same amount of drug was administered intravenously, activation of the tracing was rarely obtained; rather, on those occasions when epinephrine produced any change at all, a tendency to increase spindling was noticed. Curarized cats have been employed in a number of experiments; intravenous injection of 10-20 μg of epinephrine provoked in this animal an activation of the EEG. The injection of the drug into the common carotid artery in doses up to 10 μg was not followed by any activation; with larger doses (20-30 μg) activation of the tracing was frequently noted, but this appeared 10-20 seconds later when a rise of arterial blood pressure was observed, due to entrance of epinephrine into the general circulation.

Amphetamine. Five animals received amphetamine. No modification of the electrical tracing was observed up to 100 μg. When a cumulative dose of 200-300 μg was reached, a desynchronisation of the EEG and signs of excitement and tachypnoea were noted.

Administration of drugs into carotid artery of the "cerveau isolé" preparation. At least 1 hour elapsed between the cutting of the brain stem and the beginning of the experiment. After administration of acetylcholine up to 20 μg into the carotid artery, the activation which is obtained in the normal animal was never observed. In 4 out of 10 experiments a change in pattern was noticed, consisting in a discharge of high voltage waves at 8-12 c/sec. (Fig. 1), especially in occipital tracings. A motor reaction and tachypnoea always followed the injection.

The effects of administration of eserine and amphetamine were the same as in the normal animal; in the "cerveau isolé," however, desynchronisation was obtained only with larger doses (0.05-0.1 mg of eserine and 0.5-1 mg of amphetamine). Epinephrine was not

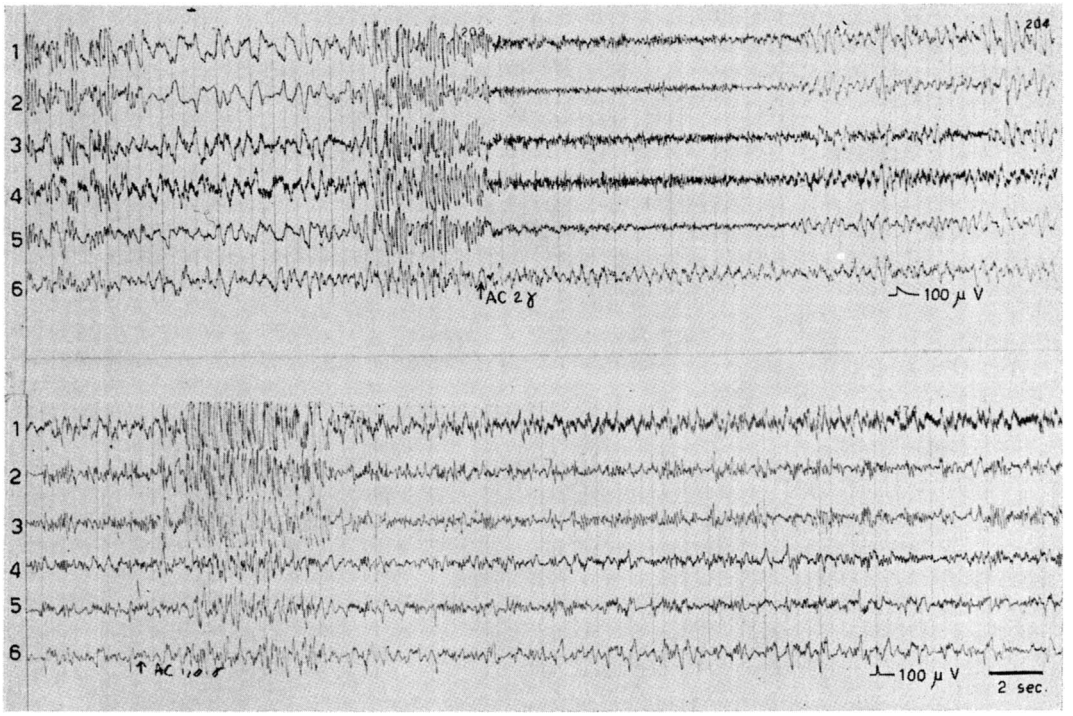

FIG. 1. Action of acetylcholine administered by intracarotid route in normal and "cerveau isolé" rabbit. *First tracing:* Rabbit 2 kg. Cannula in left internal carotid. At arrow: acetylcholine 2 μg. Activation in all leads. Searching movements of head. *Leads:* 1—L. fr.-occ.; 2—R. frontal; 3—L. fr.-R. fr.; 4—L. frontal; 5—R. fr.-occ.; 6—anteromedial thalamus. *Second tracing:* Rabbit 2, 5 kg, "cerveau isolé." Cannula in internal left carotid. At arrow: acetylcholine 5 μg (10 μg of chlorate salt). EEG response is completely different from the above and consists in discharge of high voltage waves. An intense motor reaction is present. For demonstrative purposes, a tracing of "cerveau isolé" with low voltage background was chosen. *Leads:* 1—L. occipital; 2—L. parietal; 3—L. frontal; 4—R. frontal; 5—R. parietal; 6—R. occipital.

tested in the "cerveau isolé" animals.

Discussion. The activating effect of epinephrine injected intravenously, described by Bonvallet *et al.*(1), in the cat was confirmed by the present authors; however, no activation was obtained with smaller doses by intracarotid injection. In the rabbit, the activation of EEG very rarely follows administration of epinephrine, either by intravenous or intracarotid route. DeMaar and Martin found that in the spinal vagotomized cat l-epinephrine produced activation that became increasingly feeble with repetitive doses and that synchronisation and spindling became predominant. Rothballer(16) found that in curarized cats with an intact brain stem epinephrine was not able to produce a marked activation or to completely abolish slow waves and spindling activity. In view of the results obtained in rabbits by ourselves and in cats by the forementioned authors, it appears unlikely that epinephrine plays a major role in the activation of the EEG mediated through the reticular formation.

When injected directly in the cerebral circulation, amphetamine produced an EEG response very similar to the one observed by intravenous administration of the same dose. The fact that amphetamine is still active in the "cerveau isolé" means that its action is relatively independent of the presence of the mesencephalic activating system and can be exerted on higher structures (hypothalamus).

When injected intracarotidly, eserine failed to show an action similar to ACh, *i.e.*, a desynchronisation of EEG, characterized by rapid onset and short duration. Previous experiments(Longo, 10) have shown that the

same rapid and short activation characteristic of ACh could be obtained with other substances (tetramethylammonium iodide, succinylcholine chloride, d-tubocurarine chloride). It is, therefore, suggested that whereas ACh and other substances possessing a quaternary nitrogen produce their effects by means of immediate action, the slower onset of the action of eserine is probably related to certain enzymatic changes. The difference between the effect of eserine and ACh is also evident in the "cerveau isolé" preparation. The activating action of ACh on this preparation, obtained by Rinaldi and Himwich(15), could not be confirmed. On the contrary, it appears possible to reproduce, sometimes, by means of intracarotid injections, the synchronising and convulsant effect demonstrated after topical application of the drug on the cortex(Miller *et al.*, 13; Funderburk and Case, 8). Eserine, on the contrary, is still active in producing activation in the "cerveau isolé". Desmedt and LaGrutta(5) claim that the desynchronisation, due to anticholinesterase drugs, is due to the inhibition of the cholinesterase present in the neuroglia and is not at all connected with functional variations. In this connection, it is worthwhile to remember that eserine never produces any behavioural changes, while ACh always produces searching movements and dyspnoea.

The lack of desynchronisation in the "cerveau isolé" may have some bearing upon the general problem of the locus and mechanism of the activation produced by ACh. A peripheral effect on receptors of the arterial walls or of the dura cannot be excluded. In this case, the lack of desynchronisation in the "cerveau isolé" would be due to deafferentation of the cerebrum.

It is also possible that ACh may influence the vestibular nuclei(Essig *et al.*, 7; Delgado, 4) or the respiratory centers(Gesell *et al.*, 9) or the cerebellar cortex(Crossland and Mitchell, 3) and thus, indirectly through centripetal pathways, provoke generalized activation. In fact, in the "cerveau isolé," ACh still produces motor and respiratory excitement, while the desynchronisation is not induced because of interruption of the rostral connection.

The action of ACh may be exerted directly on the cerebral cortex. In anatomical controls, the cortical layers of the cerebrum and of the cerebellum homolateral to the injected side were found stained. Only on rare occasions were the infundibular area and the dorso-anterior part of the thalamus reached; the dye was found also in the choroid plexus. The mesencephalic brain stem was never directly injected with the dye. However, since a difference in rate of diffusion between the dyes and ACh is known to exist, we must consider the anatomical findings only as orientative information.

It is known (Bremer and Terzuolo, 2) that it is possible to obtain a generalized desynchronisation after electrical stimulation of appropriate regions of the cortex, which is dependent on the cortico-reticular and reticulo-cortical circuits. The desynchronisation produced by ACh may be dependent upon reticular mechanisms initiated by cortical excitation. This hypothesis can also explain lack of action of ACh on the "cerveau isolé." In fact, Mollica(14) has demonstrated that it is impossible to provoke in this preparation the generalized desynchronisation with electrical stimulation of the cortex. Moreover, the synchronising effect, which is sometimes observed, can be caused by an action of the cortico-thalamic circuits whose activity is released from inhibitory control of midbrain reticular mechanisms.

Summary. 1) The effects on cerebral electrical activity (EEG) of acetylcholine, eserine, epinephrine, and amphetamine, injected by intracarotid route, are reported. Non-curarized unanesthetized rabbits and "cerveau isolé" preparation of the same animal were used. 2) In the unanesthetized non-curarized rabbit, acetylcholine, eserine, and amphetamine produced activation of the EEG; epinephrine, however, administered either by intravenous or intracarotid route, does not have the activating effect. 3) In the "cerveau isolé" preparation, acetylcholine does not provoke activation, while eserine and amphetamine are still effective. 4) These results are discussed in connection with the locus and the mechanism of action of the drugs.

1. Bonvallet, M., Dell, P., and Hiebel, G., *EEG Clin. Neurophysiol.*, 1954, v6, 119.
2. Bremer, F., and Terzuolo, C., *Arch. Int. Physiol.*, 1954, v62, 157.
3. Crossland, J., and Mitchell, J. F., *J. Physiol.*, 1956, v132, 391.
4. Delgado, J. M. R., *J. Neurophysiol.*, 1955, v18, 261.
5. Desmedt, J. E., and LaGrutta, G., *J. Physiol.*, 1955, v129, 46P.
6. Elkes, J., Elkes, C., and Bradley, P. B., *J. Ment. Sci.*, 1954, v100, 125.
7. Essig, C. F., Hampson, J. L., McCauley, A., and Himwich, H. E., *J. Neurophysiol.*, 1950, v13, 269.
8. Funderburk, H. W., and Case, T. J., *EEG Clin. Neurophysiol.*, 1951, v3, 213.
9. Gesell, R., Hansen, E. T., and Worzniak, J. J., *Am. J. Physiol.*, 1943, v138, 776.
10. Longo, V. G., *Experientia*, 1955, v11, 76.
11. ———, *J. Pharmacol.*, 1956, v116, 198.
12. Longo, V. G., and Silvestrini, B., *ibid.*, 1957, in press.
13. Miller, F. R., Stavraky, C. W., and Woonton, G., *J. Neurophysiol.*, 1940, v3, 131.
14. Mollica, A., *Boll. Soc. It. Biol. Sper.*, 1955, v31, 249.
15. Rinaldi, F., and Himwich, H. E., *Arch. Neurol. Psychiat.*, 1955, v43, 387.
16. Rothballer, A. B., *EEG Clin. Neurophysiol.*, 1956, v8, 603.
17. Schallek, W., and Walz, D., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 715.

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Accumulation of Organic Acids by HeLa Cells Infected with Type 4 Adenovirus.* (23113)

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That fluids from HeLa cell cultures infected with an adenovirus were more acidic than those from companion uninfected cultures was noted during early studies of the characteristics of these agents(1,2). Increased acidity of the media of infected cultures accompanied extensive cytopathogenic changes effected by the adenoviruses. Preliminary reports indicated that lowered pH of cultures infected with types 1-4 adenoviruses was a reflection of an increased quantity of lactic acid in the fluid, and the accumulation of acid was correlated with increased utilization of glucose by the infected cells(2).

This investigation was initiated to establish further the identity of acid or acids produced by uninfected and infected HeLa cells, and to determine whether an increased accumulation of acids other than lactic acid resulted from type 4 adenovirus infection. It is the purpose of this communication to report that lactic, pyruvic, acetic, and alpha ketoglutaric acids increase in fluids of HeLa cell cultures infected with this agent.

Methods. Tissue culture. HeLa cells were propagated in medium consisting of human serum, 40%, and Hanks' balanced salt solution (BSS), 60%, as described previously(3, 4). Cultures in tubes were initiated with 50,000 cells, and were used experimentally in approximately 24 hours. For infection, cultures were washed 3 times with 2 ml of Hanks' balanced salt solution per wash, and to each tube was added 0.8 ml of maintenance mixture composed of 67.5% Scherer's amino acid-vitamin mixture(5), 25% tryptose phosphate broth, and 7.5% chicken serum(4). To estimate pH of fluids, phenol red, 0.002%, was present in all media. *Viruses.* Type 4 aden-

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