TABLE IV. Therapeutic Activity of Fungicidin in Mice Infected with *H. capsulatum.*

| Infection | | Therapy | | | No. of mice, 20 g | % survival weeks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Date | No.* of microorganisms | Dates | No. of inj. | Total amt. inj. mg/kg | | 1 | 2 | 3 | 4 | 5 |
| 10/30/50 | 6,000,000,000 | 10/30/50 through 12/4/50 | 15† | 1300 | 10 | 80‡ | 80 | 80 | 80 | 80 |
| 10/30/50 | 6,000,000,000 | | 0 | 0 | 10 | 100 | 50 | 0 | 0 | 0 |

* Determined by hemocytometer.

† Mice received 9 inj. the first week, 2 the second, 1 the third, 2 the fourth, and 1 the fifth.

‡ 2 mice died within 48 hr after infection. They were thought to have been injured at time of treatment.

Infection by intraperitoneal route. Therapy by subcutaneous route. Doses ranged from 1-2 mg per mouse.

resembles actidione. The other, designated as fungicidin, is intracellular. It is both fungistatic and fungicidal and apparently lacks antibacterial action. Its activity is not diminished by the presence of horse blood or serum. Fungicidin is distinguished from other antibiotics within our knowledge by its antimicrobial spectrum and its solubility characteristics. The approximate LD 50 for crude fungicidin administered intraperitoneally to mice is between 20 and 26 mg/kg. Injected subcutaneously, its toxicity is considerably lower. Therapeutically, fungicidin appears to be of value in histoplasmosis and cryptococcosis induced in mice.

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## Concentration and Distribution of "Encephalin" in the Brain of Humans and Animals. (18398)

W. RAAB AND W. GIGEE.

*From the Division of Experimental Medicine, University of Vermont, College of Medicine, Burlington, Vt.*

The term "encephalin" designates a substance (or compound of substances?) with sympathomimetic biological action, which has been isolated from human and animal brains (1). It possesses certain epinephrine-like chromogenic and adsorbability properties, but differs from both epinephrine and nor-epinephrine in several respects. The colorimetric readings, obtained in concentrated brain dialyzates against an epinephrine standard were generally in satisfactory quantitative agreement with the pharmacodynamic action of the dialyzates (cat blood pressure, rabbit intestine). Small amounts of nor-epinephrine and epinephrine which have recently been demonstrated in brain extracts and which are believed to derive from sympathetic nerve endings in the cerebral vessels(2) are undoubtedly also present in the encephalin-containing brain dialyzates. Their calculated chromogenic effect would account for only about 1/60 of the total colorimetric readings. Various observations speak against the assumption(2) that the bulk of encephalin might be identical with tyramine. They have been discussed elsewhere(1). One of them is the fact that tyramine does not give the blue color reaction with arsenomolybdic acid which encephalin shares with the known sympathomimetic catecholamines.

Colorimetric assays of encephalin, the re-

1. Raab, W., *Am. J. Physiol.,* 1948, v152, 324.

2. Holtz, P., *Acta physiol. scandinav.,* 1950, v20, 354.

TABLE I. Average Concentrations of "Encephalin" per g (or cc) Expressed in Gamma-Equivalents (Colorimetric Epinephrine Standards).

| | Rat (271)* | Rabbit (5) | Dog (5) | Cow (15) | Bull (9) | Hog (7) | Monkey (5) | Man (16) | Human infant (6) |
|---|---|---|---|---|---|---|---|---|---|
| Brain (total) | 2.8 | 1.2 | 2.0 | 1.2 | 1.2 | 0.8 | 1.7 | 1.0 | 1.5 |
| Cortex | — | — | 1.7 | 1.5 | 1.2 | 0.8 | 1.9 | 1.2 | 1.9 |
| Corpus callosum | — | — | 1.1 | 0.9 | 0.9 | 0.8 | 1.0 | 0.7 | 1.5 |
| Thalamus | — | — | — | 0.8 | 0.9 | 0.7 | 2.1 | 1.2 | 1.5 |
| Nucl-caudatus | — | — | 3.2 | 2.9 | 4.5 | 2.2 | 5.0 | 1.9 | 2.4 |
| Cerebellum (2)* | — | — | — | 1.7 | — | — | — | 1.5 | — |
| Plexus chorioideus (4) | — | — | — | 1.3 | — | — | — | 1.3 | 2.2 |
| Pituitary gland (4) | — | — | — | — | — | — | — | 3.6 | — |
| Cisternal fluid (18) | — | — | — | — | — | — | — | 0.4 | — |
| Lumbar fluid (30) | — | — | — | — | — | — | — | 0.2 | — |

* The figures in brackets indicate the numbers of specimens examined.

sults of which are reported in the following, were carried out in different areas of the brains of humans and of several species of larger mammals, and furthermore, in the mashed whole brains of white rats under a variety of experimental conditions. Shaw's colorimetric method, as modified by one of us (3) and as adapted for brain tissue(1) was used throughout. For each experimental rat brain, a corresponding control specimen was analyzed on the same day with the same reagents. A total of 620 brains was examined.

*Results.* As shown in Table I, the highest encephalin concentrations were found in the nucleus caudatus, the lowest in the white matter (corpus callosum), with the cortex and thalamus values in between. The encephalin concentration in the nucleus caudatus of the bull was considerably higher than in that of the cow and highest of all in that of the rhesus monkey. The total brain concentration of encephalin was highest in the rat and dog, lowest in the hog and human, and higher in babies than in adult humans. Positive colorimetric readings were also obtained from the cerebellum, chorioid plexus, pituitary gland, and cerebrospinal fluid. The readings in the cisternal fluid were twice as high as those in the lumbar fluid.

The total encephalin content and its distribution were practically identical in two groups of human brains, which were somewhat arbitrarily designated as "normal" (16 specimens) and as "psychotic" (11 specimens). The average values for frontal cortex, motor cortex, corpus callosum, thalamus, nucleus caudatus and whole brain did not differ more than 0–10% between the two groups.

No major changes in the encephalin content of the brain of the rat (average 22 specimens per group; encephalin deviations from −13 to +15%) were found under the following experimental conditions:

(1) Epinephrine (10-40 mg/kg i.p.); all animals died within 5-48 minutes; (2) nor-epinephrine (40-60 mg/kg i.p.; the chromogenic intensity of nor-epinephrine is about 1/3 of that of epinephrine); died or killed within 3-20 minutes; (3) hydroxytyramine (400 mg/kg i.p.; the chromogenic effect of hydroxytyramine is about 1/70 of that of epinephrine); all killed after 30 minutes; (4) desoxycorticosterone acetate (2.5 mg i.m. daily on 8 successive days); all killed; (5) thyroxine (1 mg i.p. on 3 successive days); all killed; (6) insulin (10-40 units i.p.); all killed after 60 minutes; a second group received 20 units on 2-3 successive days; all killed 4-24 hours after last injection; (7) adrenalectomy; died or killed within 2-50 (average 19) days; (8) hypophysectomy; all killed within 82-95 days.

(9) Nembutal (6-18 mg i.p.); died or killed after 15-30 minutes; (10) morphine sulphate (20-120 mg i.p.); died or killed after 16-18 minutes.

(11) Benzedrine (10 mg i.p.); convulsions, all killed after 60 minutes; (12) metrazol (20 mg i.p.); convulsions, died or killed after 2-9 minutes; (13) strychnine (1.5 mg i.p.); convulsions, died or killed after 2-5 minutes;

3. Raab, W., *Exp. Med. and Surg.*, 1943, v1, 188.

(14) DC current through the head (10 seconds to 5 minutes); died or killed; (15) AC current through the head (Variac positions 6-32, 15-165 minutes); all died.

(16) Excitement (tied on back for 4 hours); all killed; (17) exercise (swimming in water, 30°C, 30-60 minutes); all killed; (18) low air pressure (252 mm Hg, 4 hours); all killed; (19) cold exposure (−10 to +6°C, 3-24 hours); all killed; (20) acetylcholine (under nembutal anesthesia, 15 mg in 0.2 to 0.3 cc of Ringer solution injected into a carotid artery craniad); died or killed after 1-5 minutes; (21) alcohol (daily 4 cc of absolute alcohol ingested with drinking water); died or killed after 8-24 (average 25) days; (22) N-free diet (obtained from General Biochemicals, Inc.); all killed after 23-43 days.

Highly significant changes of the colorimetric readings were observed only after i.p. injection of dihydroxyphenylalanine (300 mg/kg), namely a rise of 120% after 30 minutes, of 134% after 60 minutes, and of 66% after 120 minutes. (The chromogenic intensity of dihydroxyphenylalanine is about 1/3 of that of epinephrine.) All animals were killed.

In some of the rats which received epinephrine, hydroxytyramine or dihydroxyphenylalanine, the heart was analyzed and an accumulation of these substances was clearly demonstrable in accordance with their respective chromogenic intensity and dosage.

*Comment.* As long as nothing is known concerning the possible role of encephalin in cerebral metabolism, it would be futile to speculate on the functional significance of the pattern of its intracerebral distribution, which proved to be essentially the same in all species examined, except that in adult humans the average differences between the cortex and the brain stem ganglia were smaller than in animals. It is noteworthy that the colorimetric encephalin concentration in rabbit and cattle brain is about the same (1.1 and 1.2 γ equivalents per gram, respectively), whereas the concentration of nor-epinephrine-epinephrine has been found about 10 times higher in the brain of the rabbit than in cattle brain (0.2 γ per gram vs. 0.02 γ per gram)(2). The presence in the chorioid plexus, the pituitary gland, and the cerebrospinal fluid of material with the same adsorbability and chromogenic properties as encephalin suggests the possibility of neurosecretory discharges of encephalin from the brain into adjacent structures. In this connection, the question arises as to whether encephalin might play a similar part in the mechanism of ACTH secretion as epinephrine(4,5). The lower chromogenicity of the lumbar fluid, compared with the cisternal fluid, makes it probable that encephalin diffuses out of the spinal canal into the general circulation. Incubation of cisternal fluid at 37°C for 8 hours did not alter its colorimetric readings. The absence of any appreciable difference between the quantity and distribution of encephalin in the brains of "normal" and "psychotic" persons, although to be regarded with caution because of the somewhat arbitrary selection of cases, seems to make it unlikely that quantitative alterations of encephalin in the brain are a factor in mental disease.

The over-all concentration of encephalin in the brain of the rat proved remarkably constant under a variety of experimental procedures, such as injection of hormones, removal of endocrine glands, administration of centrally stimulating and depressing drugs, stressful situations, injection of acetylcholine, and N-free diet. The conclusion appears justified that the formation of encephalin is not dependent upon the integrity of the endocrine system, especially the adrenal glands, and that convulsions, general anesthesia, etc., occur without any gross quantitative changes of the encephalin content of the brain. However, this does not rule out the possibility of qualitative alterations of the molecular structure of encephalin which might escape detection by colorimetry. In contrast to the heart muscle, which eagerly accumulates sympathomimetic amines in large quantities, the rat brain did not retain either epinephrine, norepinephrine, or hydroxytyramine. On the

4. McDermott, W. V., Fry, E. G., Brobeck, J. R., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 609.

5. DeGroot, J., and Harris, G. W., *J. Physiol.*, 1950, v111, 12P.

other hand, injection of dihydroxyphenylalanine (Dopa) was followed by a very marked increase of chromogenicity of the brain extracts which reached its maximum within one hour and was on the decline after two hours. Whether this phenomenon is due to mere temporary deposition of Dopa in the brain or to a conversion into encephalin, is a question which deserves further study with the aid of paper chromatography.

*Summary.* Colorimetric assay of a large number of human and animal brains for "encephalin" revealed a rather uniform pattern of intracerebral distribution in various species. There was no appreciable difference between the encephalin content and its distribution in the brains of "normal" and "psychotic" humans. No major changes were observed under the influence of various hormones, adrenalectomy, hypophysectomy, convulsive drugs, electrical stimulation of the brain, narcotics, conditions of stress, acetylcholine, and N-free diet. Only the injection of dihydroxyphenylalanine was followed by a very marked temporary increase of the colorimetric readings, which suggests either a selective absorption of this substance by the brain or its conversion into encephalin.

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## Metabolism of Isomers of Linoleic and Linolenic Acids.* (18399)

RALPH T. HOLMAN. (Introduced by Carl M. Lyman.)

*From the Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station, Texas Agricultural and Mechanical College System, College Station, Texas.*

It has been repeatedly shown that the rigid exclusion of fat from the diet of rats and other animals results in cessation of growth and appearance of skin symptoms which are prevented or cured by either linoleic or arachidonic acid(1). However, either linolenic acid or hexaenoic acid permits growth, but does not cure the skin symptoms. The administration of linoleate to fat deficient rats causes the deposition of predominantly arachidonic acid (tetraenoate), and the administration of linolenic acid causes deposition of predominantly hexaenoate in certain tissues(2). However, the conversion of linoleate to hexaenoate and linolenate to tetraenoate has been demonstrated in the whole animal. Reiser has recently shown the conversion of linoleate to tetraenoate and pentaenoate, and of linolenate to hexaenoate in the chick(3). To attempt to learn something of the nature of the highly unsaturated acids deposited as the result of linoleate or linolenate feeding, a study was made of the deposition of polyunsaturated fatty acids in the rat as the result of administration of unnatural isomers of the essential fatty acids.

*Experimental.* Rats which had been kept on a fat deficient diet(2) for 10-12 months after weaning were given daily oral doses of 40 mg of ethyl esters of the various fatty acids under study for a period of 2 months. At the end of this time, the animals were killed, the total fatty acids were isolated, the polyunsaturates were estimated spectrophotometrically(4), and the total quantities of 4, 5 and 6 double bond acids were estimated as described previously(2). Ethyl linoleate and ethyl linolenate were prepared by the usual bromination-debromination procedures. Ethyl linolelaidate was prepared by selenium iso-

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