A second patient, J. McC., had a history of periodic attacks of anemia for 3 years. When the present treatment was begun he had an erythrocyte count of 2,160,000 per c. mm., hemoglobin 56% (Haskins-Sahli), color index 1.15, and reticulocytes 1.4%. He was given 9 gm. daily of nuclear extract from spleen. In 3 days the reticulocytes increased to 20%, in 6 days to 34%. Fourteen days after treatment was begun the reticulocytes had dropped again to 12%, and after 24 days to 4%, at which level they remained 31 days after treatment was begun. In the meantime the hemoglobin had increased from 55% to 100%, and the erythrocytes from 2,048,000 to 4,500,000 per c. mm., in the 31 day period.

In connection with the results obtained by Leake and Evans,[4] by McCann,[5] and by the Minot-Murphy treatment, these cases appear of interest.

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## The Digestion of Oils by Amoeba dubia.

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The following account deals with experiments consisting of injection of various oils in one of the large free-living amoebae *(Amoeba dubia)*.[1] Injection was accomplished with a Chambers micro-manipulator. The experimental amoebae were from a race which has been cultivated by one of the authors for 4 years. Thus a minimum of variability has been ensured in the amoeba protoplasm concerned. Selected individuals were isolated and injected in the usual hanging drop. They were transferred singly to depression culture dishes. The diameter of the injected oil drop was measured by a calibrated ocular micrometer. These measurements were taken daily until droplet was either extruded or completely digested. Where extruded it was invariably located in the culture medium and measured for decrease in volume. Both experimental and control animals were placed in non-toxic distilled water from the same source as that used for the mass culture of the amoebae.

Nine representative oils were used: animal, vegetable and mineral, which were chosen with reference to their suitability for this type of

[4] Leake, C. D., and Evans, J. S., *Am. J. Med. Sc.*, 1924, clxviii, 819.

[5] McCann, W. S., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 255.

[1] Schaeffer, A. A., *Arch. f. Protistenk.*, 1916, xxxvii, 204.

microinjection. All of these oils were injected in the natural condition and 4 were also injected after radiation by an ultra violet lamp (mercury arc) for 20 minutes at a distance of 24 inches. The amount of digestion accomplished in the various oils is expressed in cubic micra. The average volume of an amoeba was determined by the following method: An amoeba crawling normally was regarded as a thin cylinder. The area was computed from camera lucida drawings, at appropriate magnifications, of 20 typical amoebae. A polar planimeter was used in making the computation. The thickness of the amoeba was measured directly by placing crawling amoebae on a coverslip and viewing the animals edgeways. By this means the volume of an amoeba was found to be approximately 500,000$\mu^3$. The injected oil was considered as a sphere and the different volumes computed from the measurement of the diameter. The following table summarizes the results of the injection of this series of oils in *Amoeba dubia:*

TABLE I.

| Oil | No. amoebae injected | No. amoebae digesting oil | Aver. total vol. in $\mu^3$ digested per amoeba | Ave. vol.dig. Ave. vol. am. | No. days injected amoebae lived | No. days control amoebae lived |
|---|---|---|---|---|---|---|
| Codliver (nonradiated) | 30 | 15 | 41,300 | 8.3% | 6. | 4 to 5 |
| Codliver (radiated) | 32 | 6 | 26,900 | 5.4% | 4.5 | 4.5 |
| Olive (nonradiated) | 20 | 14 | 40,800 | 8.2% | 5.5 | 5. |
| Olive (radiated) | 29 | 24 | 11,700 | 2.3% | 5. | 5. |
| Cottonseed (nonradiated) | 23 | 16 | 20,300 | 4.0% | 6. | 6. |
| Cottonseed (radiated) | 12 | 6 | 13,400 | 2.6% | 3. | 6. |
| Linseed (nonradiated) | 11 | 0 | 0 | 0. | 5. | 5. |
| Linseed (radiated) | 16 | 4 | 8,300 | 1.7% | 5. | 5. |
| Sperm (nonradiated) | 32 | 21 | 17,500 | 3.5% | 7. | 5. |
| Peanut (nonradiated) | 19 | 9 | 6,900 | 1.4% | 3.5 | 4. |
| Nujol | 12 | 0 | 0 | 0. | 4.5 | 4.5 |
| Oleic acid | 20 | 0 | 0 | 0. | 4.5 | 4.5 |
| Oxfoot | 18 | 0 | 0 | 0. | 4.5 | 4.5 |

The relative digestibility of these oils by *Amoeba dubia* may be summarized as follows:

1. Codliver (nonradiated).
2. Olive (nonradiated).
3. Codliver (radiated).
4. Cottonseed (radiated).
5. Sperm (nonradiated).
6. Cottonseed (radiated).
7. Olive (radiated).
8. Linseed (radiated).
9. Peanut (nonradiated).
10. Linseed (nonradiated).

It appears that radiation with the ultra violet rays had a retarding effect on digestion of the oils so treated, with the single exception of linseed oil. At present we propose no explanation for this phenomenon.

The operation of injection of oil is accomplished without injury to the amoeba unless the amount injected much exceeds 225,000 $\mu^3$. When this amount of oil is injected experience has shown that the amoeba rapidly becomes sluggish, tends to round off and dies usually in 24 to 48 hours. It was found by experience that the volume of injected oil to give best results varied between volumes from about 14,000 $\mu^3$ to about 65,000 $\mu^3$ In volumes below 225,000$\mu^3$ the oil droplet was received and carried about in the endoplasm apparently in the same manner as other free-moving contained particles. An amoeba injected with an optimum volume of oil does not differ essentially in appearance, when examined soon after the injection, from a control animal and its behavior in regard to endoplasmic streaming, locomotion, etc., is strikingly similar to that of the typical control amoeba. As can be seen in Table I there is no significant difference between the total length of life of control and injected animals. In the case of nonradiated olive oil, nonradiated codliver oil and nonradiated sperm oil there is an increase in the length of life of the injected amoeba over the control.

As measurements of the oil droplets contained by the amoebas showed a decrease in diameter indicating a gradual breaking down of the oil the endoplasm correspondingly became increasingly filled with brownish particles which imparted a characteristic granular appearance to the amoeba. Such amoebae were recognizable at a glance from those which had extruded the oil without any digestion or from the control animals. Both the granular appearance and the color gradually disappeared during the course of the experiment.

Digestion did not take place in every case of injection, even with those oils in which the greatest total amount of digestion occurred

(*Cf.* Table I). The number injected represents amoebae which retained the oil for the period between injection and isolation with subsequent measurement. This period varied from 20 minutes to 2 hours.

The 3 oils last considered in Table I are similar in the respect that no digestion whatever took place. In the case of the mineral oil, nujol, none was expected. Injection of this oil was made at the beginning of the experiment to note the reaction, if any, of amoeba protoplasm to an inert fluid. When oleic acid was injected a very rapid and peculiar response followed—the oil was almost immediately surrounded by an envelope of apparently coagulated endoplasm and was extruded in times varying from 45 seconds to 3 to 8 minutes. A similar phenomenon was observed after injection of ox-foot oil but at a slower rate. (5 minutes to a few hours.)

As a result of this work definite evidence is presented that the protoplasm of *Amoeba dubia* has the ability to digest oils and therefore the presence of a lipolytic substance in this protoplasm is hereby demonstrated.

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## Cocaine Potentiation of Epinephrine and Ephedrine Action on Uterus and Intestine.

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The work of Froehlich and Loewi[1] demonstrating that cocaine increases the sensitivity to epinephrine of the iris, urinary bladder, salivary glands and blood vessels of the cat, followed by De Eds'[2] results showing antagonism by cocaine of the pressor action of ephedrine, suggested the need for work with these drugs on other organs.

With excised strips of non-gravid uterus of the rabbit, suspended in Tyrode solution, there was a definite (20-400%) potentiation of epinephrine and ephedrine actions by cocaine. The cocaine-ephedrine potentiation was evident also on the ergotaminized rabbit uterus, no longer responsive to epinephrine, and on guinea pig uterus. Ephedrine caused contraction of both organs. These facts are important

[1] Froehlich and Loewi, *Arch. Exp. Path. Pharm.*, 1910, lxii, 159.

[2] De Eds, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 551.