

vector of typhus fever has been further supported by the isolation of a strain of typhus *Rickettsia* from naturally infected mites during an epidemic. However, the importance of this vector in the transmission of murine typhus cannot be easily determined. In the present epidemic, the mite-infestation among trapped rats is decidedly low, only 4 out of 24 rats caught harbored a few mites each, and no free mite was seen around the living quarters. At the same time, the rats harbored many fleas which were proved to be infected. It is, therefore, quite possible that these mites were merely secondarily infected from feeding on already infected wild rats. The available data do not permit a decision.

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Miotic Action of Lecithin and Cephalin on Denervated Iris of Cat, Monkey, Dog and Rabbit.*

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Laked erythrocytes injected intravenously, or erythrocytes hemolyzed in the blood stream produce parasympathomimetic manifestations in the cat (Bender¹). Of these symptoms the constriction of the completely denervated iris is the most conspicuous and consistent reaction. The nature of the parasympathetic-like substance, present in the red blood corpuscles of man, monkey, rabbit, guinea pig, rat as well as in the cat is not clear. Incomplete studies made in these laboratories have suggested that the factors essential for the miosis may be associated with the phospholipids of the blood. For this reason the following comparative studies were made on the effects of certain vegetable and animal lecithins and cephalin upon the denervated iris.

Material and Methods. The animals used were cat, monkey, dog and rabbit. The iris was completely denervated by sectioning all the nerves behind the globe. Commercial preparations of lecithin and cephalin were used. Lecithin samples of vegetable, corn, soy bean, and cod fish testicle and cephalin of soy bean were supplied by the American Lecithin Company. These preparations were purified

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¹ Bender, M. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 539.

by differential extraction methods using ether extraction and reprecipitation with acetone. Artificial lecithin synthesized by method of Grün and Limpächer² in which stearic acid constitute each fatty acid radical was also tested.[†] Furthermore, lecithin from human erythrocytes was extracted and purified by modified differential extraction methods of Levene and Rolf,³ MacLean and MacLean,⁴ Bloor,⁵ and Kirk.⁶ For final testing, the phospholipid compounds were emulsified in distilled water, normal physiological saline and Locke's solutions. Volumes of one cc were injected intravenously during a period of 3 to 5 seconds. The animals were under nembutal anesthesia during the tests. In some experiments no anesthetic was used and the results were the same. The pupillary diameter was always measured with a millimeter scale.

Results. The most consistent effect obtained by the intravenous injection of the phospholipids was miosis of the cat's denervated iris. All the phospholipids mentioned above produced a conspicuous miosis in the completely denervated iris of the cat but not in that of the monkey, dog, or rabbit. The constriction occurred in every cat following the first injection and within 6 to 12 seconds after the intravenous administration of the lecithin or cephalin was begun.

Of all the lecithin studied, the synthetic preparation was the most potent; the threshold dose which produced a minimal constriction was 0.05 mg per kilo of body weight. The degree and duration of the miosis varied with the size of the dose injected; 1 mg of synthetic lecithin produced a constriction of 2 mm lasting 90 seconds, 4 mg of the same lecithin induced a constriction of 8 mm for 7 to 9 minutes. Soy bean, vegetable, corn and cod testicle lecithin and cephalin each had a miosis-inducing property, the threshold dose ranging between 0.5 mg and 1.0 mg per kilo of body weight. Intravenous injections of other colloidal emulsions and suspensions such as cholesterol (100 mg), stearic acid (10 mg), and gum acacia (60 mg) had no miotic effects.

The phospholipid miotic responses also depended on the degree of sensitiveness of the reacting organ, namely, the denervated con-

² Grün, A., and Limpächer, R., *Berichte Deutsch. Chem. Gesell.*, 1926, **59**, 1350.

[†] We are indebted to Dr. Harry H. Sobotka for a sample of synthetic lecithin which he obtained from Dr. Grün; and to Dr. Scharf of the American Lecithin Company for the commercial preparations of the lecithins and cephalin.

³ Levene, P. A., and Rolf, I. P., *J. Biol. Chem.*, 1927, **72**, 587; 1927, **74**, 713.

⁴ MacLean, H., and MacLean, I. S., *Lecithins and Allied Substances, Monographs on Biochem.*, 1927.

⁵ Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

⁶ Kirk, E., *J. Biol. Chem.*, 1938, **123**, 637.

striator fibers of the iris. Constriction of the pupil could be obtained in the acutely denervated iris but the longer the period following the post-ganglionic ciliary nerve section the more sensitive became the constrictor muscle to the phospholipids (Fig. 1). This sensitization diminished, however, when signs of regeneration appeared, usually the fifth week following the denervation.

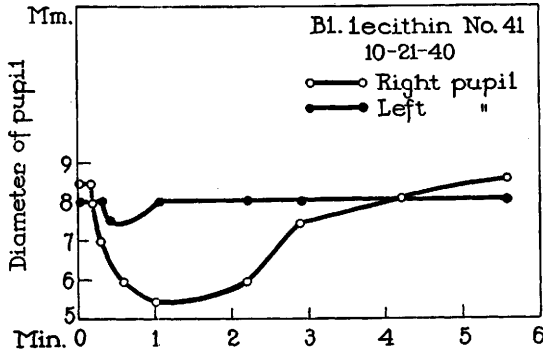


FIG. 1.

Effect of denervation on the sensitivity of the iris to phospholipids. A male cat, weighing 2.5 kilo, had its right optic and ciliary nerves sectioned on October 2, 1940. On October 19, 1940, the left optic and ciliary nerves were cut, care being taken not to injure the circulation. On October 21, 1940, under nembutal anesthesia an aqueous emulsion of vegetable lecithin, 5 mg (minimal dose for left pupil), was injected intravenously. Within 9 seconds the right pupil indicated by circles constricted rapidly while the left pupil indicated by dots constricted but slightly within 17 seconds. With a 10 mg dose the constriction became more apparent in the left pupil and more pronounced in the right. Both pupils reacted with doryl, the right constricted minimally with 1 γ , the left with 4 γ . Atropine blocked the action of doryl but not that of the phospholipid.

Atropine sulfate, injected parenterally in a dose as high as 1 mg per kilo of body weight or local application of this solution (0.1%) to the eye, had no inhibiting effect. Also, intravenous or intramuscular injections of eserine salicylate had no definite potentiating influence on the phospholipid miosis. Repeated injections of a small amount of lecithin resulted in a diminution of the miotic response with each successive dose until ultimately no miosis could be obtained (Fig. 2). Thus, when 1 mg dose is injected every 5 minutes for 6 doses the response decreases in magnitude and duration; at this point, however, the injection of 5 mg induces a miotic response which may be as great as that obtained initially.

As stated previously, none of the intravenously injected phospholipids in the concentration used with the cat and in higher concentrations had any miotic effect on the denervated iris of the monkey, dog or rabbit. Lecithin and cephalin were also without any visible effect on other denervated smooth (pupillodilator fibers) and striated muscles (facial muscles) of the cat.

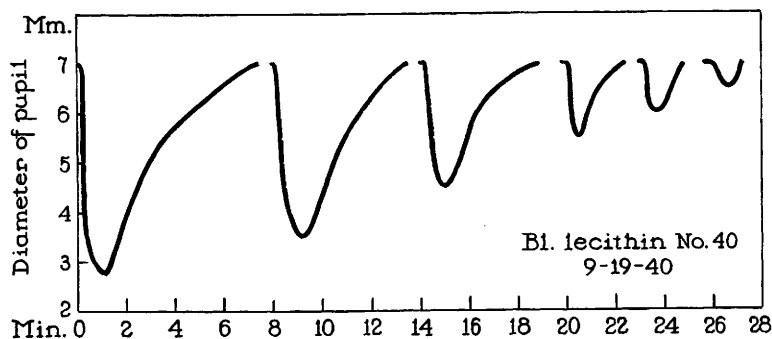


FIG. 2.

Effect of repeated intravenous injections of a constant amount of cod lecithin on the denervated iris of a cat. The denervated iris was sensitive to doryl; 1 γ produced a minimal constriction. The cat was then twice injected intravenously with 1 mg of atropine sulfate. Three drops of atropine sulfate 0.1% were also instilled in each eye. The pupil did not react with intravenous doryl as much as 100 γ per kilo after the atropinization. Cod lecithin emulsion 1 mg per kilo of body weight was then injected intravenously and the pupil rapidly constricted. Note that with each successive injection the miotic response was less in duration and degree.

Discussion. The miotic effect of the intravenously injected phospholipid emulsions is only one of a series of parasympathomimetic manifestations obtained in the cat, the other reactions not recorded in this communication being a drop in the blood pressure, slowing of the pulse rate and emptying of the bowel and bladder. These actions strongly resemble those of choline and may suggest at first glance that the reaction to phospholipid described above may be due to the hydrolyzed choline group in the lecithin. This assumption is probably incorrect for the following reasons. 1. In contrast to choline and its derivatives none of the lecithin or cephalin effect is blocked by atropine. 2. Lecithin and cephalin constrict the pupil of the completely denervated iris in the cat but not in the monkey, dog and rabbit, whereas the cholines constrict the pupil of all species. 3. Cephalin, which does not contain the choline radical, elicits a miotic reaction similar to lecithin and not influenced by atropine.

The question still remains whether the miotic effects of lecithin and cephalin are due to the phospholipids themselves or to some non-related contaminating substances. However, since synthetic lecithin is free of non-related substances which may be adsorbed by the natural phospholipids, and since this synthetic lecithin is the most potent it is reasonable to assume that lecithin itself and not some impurity is responsible for the miosis in the cat. On the other hand, a product which is used in the synthesis or possibly formed in the breakdown of lecithin such as distearoglycerophosphoric acid

(phosphatidic acid) could produce similar effects. Preliminary studies with phosphatidic acid reveal that this non-nitrogen containing phospholipid has marked mitotic properties in the cat.

The parasympathomimetic effects obtained with lecithin and cephalin, especially the miosis described, are almost identical with those produced by laked red blood cells of man, monkey, cat, rabbit, guinea pig and rat. As with phospholipids, the miosis elicited by hemolyzed erythrocytes can be demonstrated in the cat but not in other species (monkey, dog, rabbit), nor is the erythrocyte-miosis blocked by atropine. These data strongly suggest that the mitotic property possessed by erythrocytes may actually be due to the phospholipid contents.

Summary. 1. Intravenous injections of emulsions of lecithins extracted from soy bean, corn, vegetable, cod testicle and erythrocytes of man and cephalin of soy bean produce a marked miosis in the completely denervated iris of the cat but not in that of the monkey, dog or rabbit. 2. Synthetic lecithin in much smaller concentrations yields identical results. 3. None of the phospholipid mitotic effects is blocked by atropine or potentiated by eserine.

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Comparative Antitumoral Action of Desoxycorticosterone Acetate and Testosterone Propionate.*

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Uterine and other abdominal subserous fibroids which can be elicited by prolonged subcutaneous injections of estrogens fail to appear if testosterone propionate^{1, 2} or progesterone^{2, 3} is injected simultaneously with the tumorigenic estrogen. This preventive

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¹ Lipschütz, A., Vargas, L., Jr., and Ruz, O., *Lancet*, 1939, **2**, 867.

² Lipschütz, A., and Vargas, L., Jr., *Endocrinology*, 1941, in press.

³ Lipschütz, A., Murillo, R., and Vargas, L., Jr., *Lancet*, 1939, **2**, 420; Murillo, R., *Public Med. Exp.* (Chile), 1940.