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The Effect of Lecithin Administered Intravenously.

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It has been reported that various lecithins when administered by vein cause hemolysis,[1,2,3] leukocytosis,[4] changes in the fragility of the blood cellular components,[4,5,6] or death.[7] On the other hand, one investigator reports that regardless of the method of administration lecithin alone exerts no toxic action.[8] Because of the current interest in fat emulsions for parenteral use,[9,10,11] a brief study of the effect of lecithin when administered by vein has been made.

Experimental. Each sample of lecithin was prepared as a 7.0% colloidal solution in pyrogen-free distilled water and autoclaved at 10 pounds pressure for 15 minutes. The sterile solutions were rapidly injected into the marginal ear veins of normal rabbits in amounts sufficient to supply 1.75 g of lecithin per kilogram body weight. Table I shows the essential data obtained with 13 samples of lecithin. All but one of the injected rabbits died within 12 hours. These results strongly suggest that none of the tested lecithins is suitable for making fat emulsions for intravenous use.

Various methods of isolating lecithin from eggs were investigated, but the methods generally used for this purpose were not entirely successful in our hands.[12,13] The method finally adopted consisted of exhaustive extraction of powdered egg yolk with acetone to remove the fat, subsequent extraction with alcohol to obtain the crude lecithin, distillation of the alcohol under high vacuum, solution of the residue in ether and final precipitation of the lecithin with acetone. The precipitated lecithin was redissolved in ether, filtered and reprecipitated with acetone several times. Final traces of solvent were removed under high vacuum at low temperature. Yields by this method were 11% of the original weight of the egg powder.

Lecithin prepared in this manner and stored under high vacuum for varying lengths of time up to 228 days was injected into 14 normal rabbits without any apparent ill effects (Table II). The level of 1.75 g per kilo body weight was increased to 3.5 g per kilo and again without any ill effects, but 7.0 g proved to be fatal.

Samples of egg lecithin prepared and tested as above were then exposed to the air at room temperature and at 55°C for varying periods of time to determine if toxic properties would develop. As indicated in Table II, toxic properties did develop in as little as 14 days at 55°C and 47 days at room temperature. The toxicity which developed in 53 days at 55°C was profound, since both rabbits died in exactly 4 minutes after the infusions were begun. It was thought that perhaps this toxicity might be due to lysolecithin but efforts to purify the exposed sample from toxic substances by the differential solubility of lecithin

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TABLE I.
Lecithins from Various Sources.

Type of lecithin	Source*	Label description	Color	Consistency	Color of solution	No. rabbits injected	Lecithin g/kg/BW	Results
Egg	1	about 90%	dk. red	soft wax	dk. cream	2	1.75	Died in 4 and 105 minutes
"	2	—	" "	" "	" "	1	1.75	Died during night
"	3	pure	red. bk.	" "	brown	1	1.75	Died in 22 minutes
"	4	practical	brown	" "	"	1	1.75	Died in 30 minutes
"	5	pure	dk. brown	" "	"	1	1.22	Died during injection
Soybean	6	fat free phosphatides	white	granular	white	5	1.75	Died within 8 hrs†
"	6	commercial	amber	oily wax	"	1	1.75	Died during night
"	6	edible	lt. amber	heavy oil	"	1	1.75	" " "
"	6	"	dk. amber	" "	cream	1	1.75	Died in 4 hours
Animal	5	90%	brown	wax	brown	1	1.75	Died during night
Vegetable	5	60% or more	lt. amber	heavy oil	white	1	1.75	" " "
"	5	60% " "	dk. amber	" "	cream	1	1.75	" " "
"	5	app. 75%	lt. amber	hard wax	white	1	1.75	Complete paralysis recovered in 1 wk

* (1) Merck & Co.; kindly supplied by Dr. L. Emmett Holt, Jr.; (2) Difco Laboratories, Inc.; (3) Coleman and Bell Company; (4) Eastman Kodak Co.; (5) Pfanstiehl Chemical Company; (6) American Lecithin Company; kindly supplied for research by Dr. A. Scharf.
† One died in 7 min.

TABLE II.
Experimental Lecithin Preparations.

Method of preparation or treatment	Color of lecithin	Color of solution	No. rabbits injected	Lecithin g/kg/BW	Results
Acetone ppt'd from ether solution	amber	white	14*	1.75	No apparent ill effects
" " " " "	"	cream	1	3.5‡	" " " "
" " " " "	"	"	1	7.0‡	Died during night
Exposed to air at room temp. 47 days	dk. red	brown	2	1.75	One died during the night; one no apparent ill effects
" " " " " 86 "	brown	"	2	1.75	" " " " " " " " " "
" " " at 55°C 14 days	dk. red	"	2	1.75	One no apparent ill effects; one died during week-end, 72-120 hours
" " " " " 53 "	black	dk. brown	2	0.625	Both died 4 min. after start of inj.
Cadmium chloride pptn.[12]	amber	white	1	1.75	Died during night
Cadmium chloride pptn.†	"	"	1	1.75	No apparent ill effects

* Several of these rabbits were injected 2 or more times.
† Cadmium removed by pptn. with H_2S.
‡ In 14% solution.

and lysolecithin in ether[14] were unsuccessful.

Chemically speaking, the purest lecithin has been prepared through the cadmium salt.[12] This procedure did give a very light-colored and attractive preparation, but a fat emulsion prepared with it was promptly fatal to a dog and the lecithin itself was fatal to a rabbit (Table II). The toxicity of lecithin prepared in this manner was found to be due to traces of cadmium which could be removed by treatment with hydrogen sulphide.

Summary. Thirteen samples of commercial lecithin were fatal to rabbits when administered intravenously at a level of 1.75 g per kilo body weight.

A method for preparing lecithin from egg yolk is described and it is shown that lecithin so prepared is harmless to rabbits when given by vein even at a level of 3.5 g per kilo body weight. Lecithin so prepared will acquire toxic properties if exposed to the air. Elevated temperatures accelerate this process. If the lecithin is stored under high vacuum, toxic properties will not develop within at least 228 days.

Egg lecithin prepared through the cadmium salt may contain sufficient quantities of cadmium to produce cadmium poisoning.

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In vitro Susceptibility of *Histoplasma capsulatum* to Therapeutic Agents.

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During treatment of 3 cases of histoplasmosis in the early months of 1945, it was decided to carry out *in vitro* susceptibility studies on the yeast phase of *Histoplasma capsulatum*.

Methods. Five-day-old cultures of *Histoplasma capsulatum* in heart-brain infusion broth (Difco) were used as the test organism.

The drugs tested were neoarsphenamine, neostam, sulfadiazine, sulfathiazol, stilbamidine.* Two antibiotics, streptomycin* and penicillin were likewise tested. The drugs were added to the media, before inoculation of the organism, in the following mg% concentrations: 100, 50, 25, 20, 10, 1, 0.1, 0.01, and 0.001.

The streptomycin was employed in the same manner, but in concentrations of 2500, 1250, 750, 250, 125, 100, 75, 50, 25, 12.5, 10, 7.5, and 5 units per ml. Penicillin was used in concentrations of 10, 5, and 1 units per ml.

Culture tubes of heart-brain infusion at pH 7.4, containing the desired concentrations of drugs in 10 ml, were inoculated, in duplicate, with 0.5 ml of a 5-day broth culture of the yeast phase of the test organism and incubated at 37°C. These cultures were examined for growth at the end of 7 days of incubation. Similar tests were performed in which the amount of inoculum was reduced to 0.2 ml of the 5-day culture of *Histoplasma capsulatum*. Each of these series, in duplicate, was repeated once with identical results.

A control series, in which drug-free culture medium was inoculated with the same quantity of inoculum as used in the drug series, was performed with each experiment.

The cultures were examined at the end of 7 days, and subcultures were made from all tubes which did not show growth or which were very cloudy. In tests using neoarsphenamine, stilbamidine, and streptomycin, some precipitate was formed when these agents were added to the heart-brain infusion broth. In the case of streptomycin, this was believed to be due to lipoid substances present in the streptomycin. In the case of stilbamidine,

* The stilbamidine and streptomycin used were kindly supplied by Merck and Co.