at a rate of 2 to 2.5 cc per minute, with a gas mixture of 95% oxygen and 5% carbon dioxide bubbling through the fluid in the cannula.

The ascorbic acid determinations were made with the 2,6-dichlorindophenol method. The approximate concentration of hydrogen peroxide was determined by its luminescent reaction with 3-aminophthalhydrazide.[5,1]

Results. Without inhibitor, solutions of *l*-ascorbic acid with an initial concentration of 1:10,000 (in Ringer solution not specifically freed from copper[6]) caused systolic standstill within one to 2 hours, 80 to 90% of the *l*-ascorbic acid being destroyed within this period. Hydrogen peroxide accumulated in the solutions to reach concentrations of the order of 1:100,000 to 1:300,000.[1] Serum globulin or sodium diethyldithiocarbamate in appropriate concentrations inhibited the destruction of *l*-ascorbic acid and the solutions had little or no action upon the heart (Table I). The concentration of hydrogen peroxide was very low in the solutions in which the ascorbic acid destruction was markedly inhibited. The characteristic luminescence with 3-aminophthalhydrazide was either very weak or entirely absent, indicating concentrations of hydrogen peroxide of only one in 5 to 10 million.

These experiments show that *l*-ascorbic acid solutions (in the presence of oxygen and at pH 7.5 to 7.8) do not exhibit their characteristic positive inotropic and systolic effect upon the frog heart in the presence of copper inhibitors. Under this condition the hydrogen peroxide formation in the solution is insufficient to cause the heart effect.

The author is indebted to Dr. Sydney Ellis for the *l*-ascorbic acid estimations.

5 Schales, O., *Ber. d. deutsch. Chem. Ges.*, 1938, **71**, 447.

6 Peugnet, H. B., *Science*, 1939, **90**, 162.

14182

Preparation of and Cardiovascular Response to Neo-synephrine in Oil.

A. M. LANDS AND C. W. GEITER.

From the Pharmacological Research Laboratory, Frederick Stearns and Company, and the Department of Pharmacology, Wayne University School of Medicine, Detroit, Mich.

Neo-synephrine HCl (laevo a-hydroxy-b-methyl-amino-3-hydroxy ethyl benzene hydrochloride) when used parenterally has been found to be a useful adjuvant in spinal anesthesia[1,2,3,4] and in cyclopropane anesthesia.[5] This substance raises and maintains blood pressure over a considerable period of time. Side reactions such as nervousness, anxiety, etc., are comparatively rare. In addition, neo-synephrine HCl has been used successfully in the treatment of some forms of shock.[6,7]

Blood pressure within a normal range can be maintained under various conditions by repeated intravenous injections of small amounts of neo-synephrine HCl, or by subcutaneous or intramuscular injections repeated as necessary. The latter methods of administration tend to establish a depot from which absorption takes place rather slowly into the blood stream. Inasmuch as substances in oil, when injected parenterally, are absorbed more slowly than those in aqueous solutions, the

1 Lorham, P. H., and Oliverio, R. M., *Anesth. and Analg.*, 1938, **17**, 44.

2 Brunner, R. S., and deTakats, G., *Surg., Gyn., and Obst.*, 1939, **68**, 1021.

3 Bitterich, N. M., *Anesth. and Analg.*, 1939, **18**, 29.

4 Cranston, Elizabeth M., and Bieter, R. N., *J. Pharm. Exp. Therap.*, 1940, **68**, 141.

5 Orth, O. S., Leigh, M. D., Mellish, C. H., and Stutzman, J. W., *J. Pharm. Exp. Therap.*, 1939, **67**, 1.

6 Mahaffey, H., *Anesth. and Analg.*, 1939, **18**, 196.

7 Johnson, Carl A., *Surg., Gyn., Obst.*, 1937, **65**, 458.

TABLE I.
Toxicity of Neo-Synephrine HCl and Neo-Synephrine in Oil Compared.

Drug	Dose mg/kg*					
	30	35	40	45	50	55
Neo-Synephrine HCl	5/30†	10/30	14/30	21/30	27/30	—
Neo-Synephrine in Oil	—	8/30†	9/30	14/30	20/30	25/30

* All injections were made subcutaneously into albino rats. Survival was recorded for 4 days. The LD 50 was obtained by plotting the Log Dose against % mortality.
† No. dead/ No. injected.

intramuscular injection of neo-synephrine in oil should result in a more prolonged vasoconstriction. Accordingly, we have prepared and are reporting the results obtained with such a solution.

Methods. Neo-synephrine base, insoluble in oil, was found by Heyn and Geiter[8] to be soluble in peanut oil containing oleic acid. The solution employed in this investigation contained 0.82% neo-synephrine base dissolved in peanut oil with 10% oleic acid as the solubilizing agent. The amount of base present is equivalent to that contained in a 1% neo-synephrine HCl aqueous solution.

The effects on blood pressure and heart rate were determined on the unanesthetized dog following subcutaneous and intramuscular injections. Blood pressure was measured by means of a mercury manometer to which was attached a cuff specially designed to fit snugly the upper foreleg or thigh. In all instances, the response to the injection of neo-synephrine in oil was compared with that resulting from the injection of an equivalent amount of an aqueous neo-synephrine HCl. The toxicity was determined by subcutaneous administration to albino rats. All experimental animals were housed in air-conditioned quarters with a constant temperature of 75°F and a relative humidity of 35%. Thirty animals with an average weight of 200 g and of equal sex were injected at each dosage level. Neo-synephrine HCl as a 1% aqueous solution was injected in the following amounts: 30, 35, 40, 45, and 50 mg/kg. Neo-synephrine in a 1% equivalent oil solution was injected in the following amounts: 40, 45, 50, and 55 mg/kg.

Results. In the trained dog, atropinized and without an anesthetic, a definite rise in blood pressure was produced with as little as 0.05 mg/kg of neo-synephrine HCl or an equivalent amount of neo-synephrine in oil. Intramuscular injections of 0.1-0.25 mg/kg of neo-synephrine in oil was followed by a rise more sustained than that caused by an equal amount of aqueous neo-synephrine HCl. Whereas the latter solution in the above amounts, caused rises in pressure lasting as long as 90 minutes, those in oil caused rises lasting as long as 197 minutes. Injections of 0.5 mg/kg of either solution were not well tolerated and frequently caused such reactions as:

1. Pilomotor action over shoulders and tail.
2. Distinct hyperpnea.
3. Occasional emesia near or during the peak of the blood pressure rise.

Generally these reactions were associated with blood pressure elevations of approximately 75 to 130 mm Hg above the normal resting systolic pressure.

In the unatropinized dog, there is a marked bradycardia simultaneous with the rise in blood pressure, particularly in the diastolic pressure. With near threshold doses, cardiac slowing may be the only demonstrable response. The administration of neo-synephrine in oil leads to a more prolonged response than does an equivalent amount of neo-synephrine HCl by either subcutaneous or intramuscular routes. Thus, neo-synephrine HCl injected subcutaneously in doses of 0.1 mg/kg produced rises in systolic pressure up to 70 mm Hg with elevations lasting as long as 225 minutes. Under the same conditions neo-synephrine in oil caused rises up to 64 mm Hg with the duration of some elevations lasting as long as 340 minutes. Intramuscular injections gave results similar to those cited above with the exception that the duration of the elevated pressure was somewhat more

[8] Heyn, M., and Geiter, C. W., unpublished data.

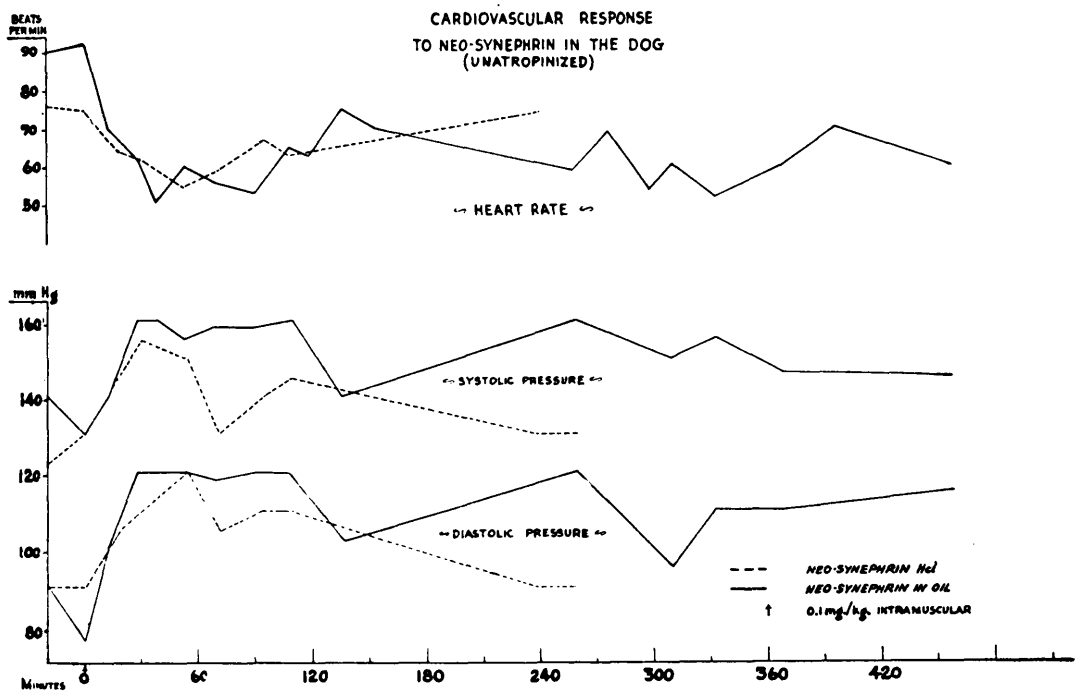

FIG. 1.

prolonged in most instances. The cardiac slowing was more prolonged than the rise in blood pressure following the injection of neo-synephrine in oil by either parenteral route. Fig. 1 gives in some detail the comparative responses obtained in one animal. This is representative of the series.

When administered subcutaneously in a series of albino rats the LD 50 of the neo-synephrine in oil is 45.9 mg/kg. The LD 50 of the neo-synephrine HCl under similar conditions is 40.7 mg/kg (equivalent in terms of neo-synephrine base to 33.4 mg/kg) (Table I). Therefore toxicity of the neo-synephrine in oil would be 28% less than that of the aqueous hydrochloride.

Summary. 1. Neo-synephrine base is soluble in peanut oil containing oleic acid. 2. Neo-synephrine in oil injected subcutaneously or intramuscularly brings about a more sustained rise in blood pressure than does a similar injection of an equivalent amount of aqueous neo-synephrine HCl. 3. The threshold dose for both forms of neo-synephrine is about 0.05 mg/kg in the dog. Maximal responses are usually obtained with doses of 0.5 mg/kg by either subcutaneous or intramuscular injection. The latter dosage level is not well tolerated and is frequently followed by undesirable side reactions. 4. Neo-synephrine in oil is 28% less toxic than the aqueous neo-synephrine HCl equivalent. The slower rate of absorption from oil is the probable cause for the lower toxicity.