pressure of an individual male rat during diethylstilbestrol injection was 11 mm Hg. above the preinjection level and of a female rat 19 mm Hg. These variations are within the normal range for the rat.

There was, however, a slight difference between the blood pressures of the male and female groups. This is illustrated in Fig. 1, in which the blood pressures taken each day on the 5 animals in each group are averaged and plotted. The males are plotted under (A), and the females under (B). It is evident that the male rats receiving cottonseed oil had slightly lower blood pressures than the control rats or those receiving diethylstilbestrol. It is also evident that the blood pressures of the female rats receiving diethylstilbestrol are somewhat higher than the control or cottonseed oil-treated groups. The highest blood pressure readings were observed about the 10th day. Blood pressures obtained at intervals of several days and continued up to 54 days after the period of injection revealed no further change.

*Comment.* These experiments have yielded results that are in accord with the report of Matthews, Emery and Weygandt[4] that hypertensive levels of blood pressure do not occur in rats during continuous treatment with diethylstilbestrol. However, upward trends of blood pressure, not observed by these investigators, were observed in the present experiments. Hypertension of the degree reported by others[1-3] was not observed. Cottonseed oil when used alone slightly reduced the blood pressure of male rats, but did not affect the pressures of female rats. It, therefore, does not explain the slight elevation observed in the female rats treated by diethylstilbestrol in cottonseed oil.

*Summary.* Diethylstilbestrol did not induce hypertension in normal rats during 24 days of continuous intramuscular injection. Moderate but transient elevations of systolic blood pressure occurred in female but not in male rats.

## 15637

## Some Cross-Circulation Experiments with Di-$\beta$-Chlorethyl Sulfide Poisoned Dogs.

SIMON BLACK AND JOHN F. THOMSON. (Introduced by E. M. K. Geiling.)

*From the Toxicity Laboratory* and the Department of Pharmacology, University of Chicago.*

Death of animals caused by di-$\beta$-chlorethyl sulfide (mustard gas) has been considered by some workers[1] to be due to the liberation from poisoned tissues of a "secondary toxin." We have attempted to test this hypothesis by cross-circulation experiments with dogs.

When cross-circulation between a poisoned and an unpoisoned dog was begun after the original poison had disappeared from the blood of the first member, no symptoms of toxic action were evident in the second animal during a subsequent observation period of 15 days. The dose used was about 30 times the $LD_{50}$ dose.

*Experimental. Animals.* Mongrel dogs of both sexes weighing 8-10 kg were used.

*Cross-circulation.* The animals were anesthetized with 30 mg/kg of nembutal intraperitoneally, and given additional small doses intravenously as required during the experiment. The left external jugular vein and left common carotid artery, of one dog, and the corresponding vessels on the right side of the other, were exposed. The 2 animals were then strapped together so that their exposed vessels could be conveniently joined. The distal end of the exposed portion of each vessel was ligated and the proximal end clamped with a bull-dog clamp. The ex-

* This work was done under contract with the Medical Division of the Chemical Warfare Service.

1 Lewis, T. L., and Grant, R. T., *Heart*, 1924, **11**, 209.

TABLE I.
Hematocrit and Plasma Glucose Levels in Blood of Dogs in Cross-Circulation Experiments.
Poisoned animal given 80 mg/kg of mustard* intraperitoneally.

| Exp. No. | Survival time of poisoned animal† Hr. Min. | Time after poisoning at which cross-circulation began Hr. Min. | Duration of cross-circulation Hr. Min. | Hematocrit (%) | | | | | Plasma glucose (mg %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Poisoned animal: initial value | Poisoned animal: at beginning of cross-circulation | Unpoisoned animal: at beginning of cross-circulation | Poisoned animal: at termination of cross-circulation | Unpoisoned animal: at termination of cross-circulation | Poisoned animal: initial value | Poisoned animal: at beginning of cross-circulation | Unpoisoned animal: at beginning of cross-circulation | Poisoned animal: at termination of cross-circulation | Unpoisoned animal: at termination of cross-circulation |
| 1 | 4 : 15 | 1 : 00 | 2 : 00 | 36 | 49 | 40 | 56 | 56 | 106 | 123 | 130 | 196 | 192 |
| 2 | 2 : 10 | 0 : 35 | 0 : 45 | 39 | 48 | 38 | 61 | 61 | 115 | 146 | 98 | 230 | 138 |
| 3 | 4 : 10 | 0 : 30 | 3 : 00 | 47 | 53 | 32 | 60 | 59 | 135 | 232 | 112 | 228 | 249 |

* Given as 10% solution in propylene glycol.
† The unpoisoned animals survived beyond a 15-day observation period.

posed artery of each animal was then joined to the vein of the other by means of U-shaped Pyrex glass cannulae with inside diameter of about 3 mm. These cannulae were thoroughly cleaned and dried, and filled with heavy mineral oil before insertion into the blood vessels in order to minimize the danger of intracannular clotting. When it was desired to begin cross-circulation the bull-dog clamps were removed from the vessels. When the experiment was to be terminated the vessels were ligated, the cannulae removed, and the incision closed with wound clips.

The rapidity of exchange of blood by a pair of cross-circulating animals was indicated by a test with Evans Blue dye. A solution of this dye was injected intravenously (fore leg) into one member of a cross-circulating pair one hour after cross-circulation was begun. Two minutes after the injection the concentration of the dye in the venous plasma of the second dog was equal to that in the plasma of the first one.

*Results. Injection of poison after cross-circulation was begun.* In one experiment a 100 mg/kg dose of mustard was injected intraperitoneally into one member of a cross-circulating pair, and the cross-circulation terminated 30 minutes later. The poisoned dog died within 3 hours. The second dog died within 4 days. Pathological findings† in the second dog were typical of severe intravenous mustard poisoning and included widespread hemorrhages of the lungs, pulmonary edema, gangrene of the right lung, and splanchnic hyperemia. The thymus was markedly atrophied.

*Injection of poison before beginning cross-circulation.* Several experiments were done in which cross-circulation between 2 animals was not begun until 30-60 minutes after one member of a pair was given an intraperitoneal dose of 80 mg/kg of mustard. In all cases the hematocrit values rapidly became identical, or nearly identical, in the 2 animals. The plasma glucose levels also usually rose to approximately the same value (Table I). Despite this evidence of a large exchange of blood between the cross-circulating animals, and the fact that all poisoned members died within 5 hours of poisoning, all of the unpoisoned members survived the experiment. There was no evidence of toxic action, such as loss of appetite or excessive thirst in the unpoisoned members of the cross-circulating

† Autopsy was performed by Dr. C. C. Lushbaugh.

pairs.

*Discussion.* The results of these experiments indicate that when a large dose of mustard is given intraperitoneally it rapidly enters the blood stream in considerable quantity. Within 30 minutes, however, it has disappeared from the circulation. This confirms chemical determinations of mustard in the blood of poisoned dogs.[2]

The survival of the unpoisoned members of cross-circulated pairs when cross-circulation began 30-60 minutes after poisoning indicates that no large amount of any "secondary toxin" was present in the circulation after the original poison had disappeared from the blood. It should be emphasized that the dose of the poison was many times larger (80-100 mg/kg) than the $LD_{50}$ dose. If production of a "secondary toxin" was proportional to the dose of the original agent, a relatively small exchange of blood between a poisoned and an unpoisoned animal should have caused death in the second animal. We must conclude, therefore, that death by acute mustard poisoning does not result from liberation of a "secondary toxin" which is freely diffusible into the blood stream.

When smaller doses of the poison are used death is delayed for several days. The cross-circulation technic is not suited to determining whether a "secondary toxin" is involved in such cases.

*Summary.* 1. A large intraperitoneal dose of di-$\beta$-chlorethyl sulfide was given one member of a cross-circulating pair of dogs. Death was produced in both animals, though it was much delayed in the second member. 2. Cross-circulation between 2 dogs was begun 30-60 minutes after poisoning one with a large intraperitoneal dose of mustard. In no case was death produced in the second, unpoisoned dog. 3. It is concluded that a "secondary toxin" is not responsible for death in acute mustard poisoning.

[2] Thomson, J. F., unpublished.

## 15638 P

## Effect of Activity on the Visco-Elasticity of Normal and Iodoacetate Muscles.

ALEXANDER SANDOW AND MANFRED BRUST. (Introduced by Harry A. Charipper.)

*From the Department of Biology, Washington Square College of Arts and Science, New York University.*

Gasser and Hill's studies[1] on the visco-elasticity of muscle by the damped vibrating spring method have been questioned in subsequent work.[2-4] The present research employs the spring technic, but criticisms of it have been largely overcome by eliminating slack of the muscle at all times, by holding constant the initial length of any muscle subjected to a series of tests, and by limiting visco-elastic comparisons to either states of contraction or rest as modified by previous activity.

Excised, Ringer's-equilibrated *Rana pipiens* sartorii were used throughout. The muscles (average excised length, 34.8 mm) were stretched 15% when attached to the undeflected spring, and a further 15% stretch occurred with the spring maximally deflected.

In the work reported below, each plotted value represents the average behavior of at least 3 different muscles, and, in general, the mean deviation for the viscosity coefficient

[1] Gasser, H. S., and Hill, A. V., *Proc. Roy. Soc., B*, 1924, **96**, 398.

[2] Hogben, L. T., and Pinhey, K. F., *Br. J. Exp. Biol.*, 1926, **4**, 196.

[3] Steinhausen, W., *Pflüg. Arch. f. d. ges. Physiol.*, 1926, **212**, 31.

[4] Schoepfle, G. M., and Gilson, A. S., Jr., *J. Cell. and Comp. Physiol.*, 1946, **27**, 105.