terial, presumably "fibrinoid," is necessary for the accumulation of carbon particles in the glomerular capillaries during the development of the GSR clearly indicates that particle retention is the primary result of adherence rather than altered capillary permeability or vasodynamic changes.

By outlining the usually inconspicuous early "fibrinoid" deposits, carbon particle retention in the glomerular tuft is a useful parameter of the initial stages of "fibrinoid" formation in the GSR. Separate evaluation of the mechanisms of particle retention at other sites will be necessary since differences in vascular architecture and function may result in variations in the method of particle capture.

Summary. During the development of the generalized Shwartzman reaction elicited by colchicine in the pregnant Syrian hamster, intravenously administered carbon particles are trapped in the renal glomeruli. The carbon particles are attached to underlying deposits of an amorphous material within the glomerular capillaries. It is concluded that this material is responsible for the capture of circulating carbon particles.

1. Galton, M., Science, 1963, v139, 923.
2. ———, Am. J. Path., 1964, v44, 613.
3. McKay, D. G., Rowe, F. J., Lab. Inv., 1960, v9, 117.
4. Majno, G., Palade, G. E., J. Biophys. Biochem. Cytol., 1961, v11, 571.
5. Pappas, G. D., Ross, M. H., Thomas, L., J. Exp. Med., 1958, v107, 333.
6. Ryter, A., Kellenberger, E., J. Ultrastruct. Res., 1958, v2, 200.

Received May 3, 1965. P.S.E.B.M., 1965, v119.

Effect of Host Weight Loss on Friend Leukemia Virus Infections in Swiss, DBA/2, and BALB/c Mice.* (30398)

ROBERT W. SIDWELL, GLEN J. DIXON, SARA M. SELLERS, CHARLOTTE F. MAXWELL, AND FRANK M. SCHABEL, JR.
Virus Section, Southern Research Institute, Birmingham, Ala.

To evaluate potential antiviral agents properly, particularly those used against oncogenic viruses, it is important to consider all possible factors which may influence the results of experiments with these agents. A primary criterion for evaluation of these drugs is to determine the inhibition of the virus-induced tumor. The effect of host weight loss on such a neoplasm could therefore be of considerable importance, especially when nonlethal drug toxicity can result in decreased food consumption and subsequent host weight loss or failure of the host to gain weight. Weight loss induced by caloric restriction has been shown to cause inhibition of growth of many experimentally induced and spontaneous tumors(1).

Among the known oncogenic viruses, the leukemia virus of Friend (FLV) has been used recently for several drug evaluation studies(2,3,4). One advantage of using this virus in chemotherapy experiments is that it induces a marked, reproducible splenomegaly in Swiss, DBA/2, and BALB/c mice following a latent period of less than one week(5,6). Because of this early onset of overt disease symptoms and the repeated use of the virus in chemotherapy experiments, FLV was chosen for the present studies of host weight loss effects.

This report presents quantitative studies on the effects of caloric restriction and resultant host weight change on the development of splenomegaly induced by FLV. In addition, the effect of host weight change on the amount of virus detectable in the spleens and plasma of FLV-infected mice has been studied. The anti-FLV activity of 2 compounds, porfiromycin and 1-methyl-1-nitrosourea, was evaluated using the conclusions drawn from these studies.

Materials and methods. DBA/2 and

* Supported by CCNSC, NCI, NIH Contract PH-43-63-68.

BALB/c mice were obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Random-bred Swiss mice were obtained from Southern Animal Farm, Prattville, Ala. All animals were 6 to 8 weeks old, of a single sex, and weighed 18-21 g at time of virus inoculation. Friend virus (spleen filtrate from the 154th passage in Swiss mice) was obtained from Dr. H. Christine Reilly, Sloan-Kettering Inst., Rye, N. Y. It was passed 5 times through Swiss mice in our laboratory prior to use. Stock virus was prepared by homogenizing spleens in a sucrose-buffer solution(9) to make a 10%, weight-to-volume, suspension. This homogenate was centrifuged at 2000 rpm in an International clinical centrifuge for 10 minutes and the supernate passed through sterile Selas 03 candle filters. The filtrate was stored in sealed ampules at *ca* —70°C until use.

To determine the effect of host weight loss on FLV infections, 50 mice of each strain were inoculated intraperitoneally (i.p.) with 0.2 ml of spleen filtrate containing approximately 10^4 Swiss mouse ID_{50} of virus. The animals were then randomly distributed into groups of 10 mice each. Each mouse was weighed, caged separately, and given an accurately weighed amount of powdered Purina laboratory chow daily, according to group. Each succeeding day any remaining food was discarded and replaced with a newly weighed amount. The following food allowances were given: *ad libitum,* 5.0, 4.0, 3.0, or 2.0 g per mouse per day.

The mice were observed daily, and on the 14th day after virus inoculation all were again weighed and the weight changes recorded. The animals were sacrificed and the spleen from each was removed and weighed. A portion of the plasma from each animal was aseptically removed also. The approximate virus titers of the spleens and heparinized plasma were determined by splenomegaly assay in indicator Swiss mice inoculated i.p. with serial $\log_{10}$ dilutions of the Selas 03 filtered material.

As controls for these studies, normal, uninfected mice of each strain were held for 14 days on the same diets as the virus-infected mice. Spleens and plasma of these control animals were then collected and bioassayed for virus as described above. An average spleen weight of 140 mg was obtained from indicator mice inoculated with spleen or plasma filtrates from these animals.

The average host body weight change for each group of mice was determined by subtracting average original weight from average final weight. A host body weight change difference was then calculated by subtracting average host body weight change of animals fed a restricted diet from average weight change of mice allowed to feed *ad libitum.* If an inhibition of splenomegaly occurred in association with body weight loss, the percent of this inhibition per gram of host weight difference was determined by dividing the percent of inhibition by the host weight difference. An average splenomegaly inhibition per gram of host weight difference was obtained from the latter data.

Results and discussion. Three experiments were carried out with each strain of mice. The results of these studies in Swiss, DBA/2, and BALB/c mice are summarized in Tables I-III.

Loss of body weight in Swiss mice had a marked effect on FLV-induced splenomegaly. Virus-infected mice fed 2 g of food per day had spleens of normal (uninfected) size. In this strain of mice the average splenomegaly inhibition per gram of host weight difference was 13.6% (Table I). The amount of demonstrable virus in the spleens and plasma of these animals varied, but no relationship was observed between body weight change and virus titer nor between average spleen weight and virus titer. These results, in regard to Swiss mouse splenic tissue and virus yield, differ with those reported by Rowe and Brodsky(7), but, as pointed out by these investigators, the virus yield from FLV-infected spleens could only be a function of mean log spleen weight if no marked physiological changes occur in the mouse.

Similarly, splenomegaly induced by FLV in DBA/2 mice was depressed by concomitant body weight loss. The average splenomegaly inhibition per gram of host weight difference was *ca.* 12.4% (Table II). The titers of virus recoverable from plasma and

TABLE I. Effects of Caloric Restriction on Friend Leukemia Virus-Induced Splenomegaly and Infectivity of Spleens and Plasma in Swiss Mice.

Exp	Food allowance (g/mouse /day)	Host body wt change* (g) Avg	Range	Difference (*ad lib* - restricted)	Spleen wt Avg (mg)	% of *ad lib* controls	% Splenomegaly inhibition per g host weight difference	Mortality	Virus titer† Spleen	Plasma
1	*Ad lib*	+4.4	−1.5, +10.5		834			0/10	—	—
	5.0	+2.1	−1.5, +7.0	2.3	696	84	7	0/10	—	—
	4.0	+1.4	−2.0, +3.0	3.0	681	82	6	0/10	—	—
	3.0	−2.0	−3.5, − .5	6.4	228	27	11	0/10	—	—
	2.0	−4.9	−6.0, −3.0	9.3	181	22	8	1/10	—	—
2	*Ad lib*	+2.7	− .5, +6.5		861			1/10	10^{-1}	10^{-3}
	5.0	− .2	−3.0, +2.0	2.9	827	96	1	1/10	10^{-1}	10^{-3}
	4.0	−1.3	−4.0, .0	4.0	565	66	9	0/10	10^{-1}	10^{-1}
	3.0	−4.0	−6.0, −1.0	6.7	384	45	8	0/10	10^{-3}	10^{-3}
	2.0	−4.5	−6.0, −3.0	7.2	188	22	11	2/10	10^{-3}	10^{-1}
3	*Ad lib*	+2.6	−1.5, +5.0		934			0/10	10^{-4}	10^{-4}
	5.0	+1.0	−3.0, +4.0	1.6	720	77	14	0/10	10^{-3}	10^{-5}
	4.0	− .1	−4.0, +2.3	2.7	724	77	9	0/10	10^{-4}	10^{-3}
	3.0	−2.7	−6.2, − .5	5.3	354	38	12	2/10	10^{-3}	10^{-3}
	2.0	−4.6	−6.2, −2.5	7.2	131	14	12	1/10	10^{-4}	10^{-4}
							Avg = 13.6			
							S.E.‡ = ±2.0			

* Host body weight change = the difference in host weight on day of virus inoculation and day of sacrifice.

† Virus titer is expressed as the maximum dilution of Selas 03-filtered spleen suspension or plasma that will induce an average spleen weight of 400 mg in indicator Swiss Mice.

‡ S.E. = Standard error as determined by *t* test.

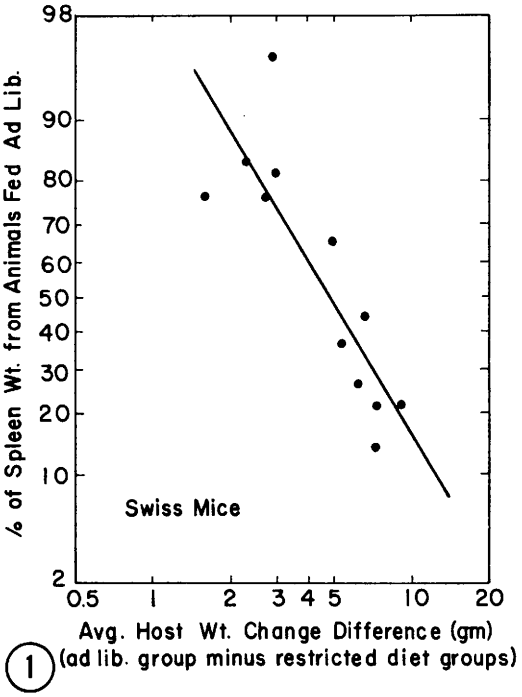
TABLE II. Effects of Caloric Restriction on Friend Leukemia Virus-Induced Splenomegaly and Infectivity of Spleens and Plasma in DBA/2 Mice.

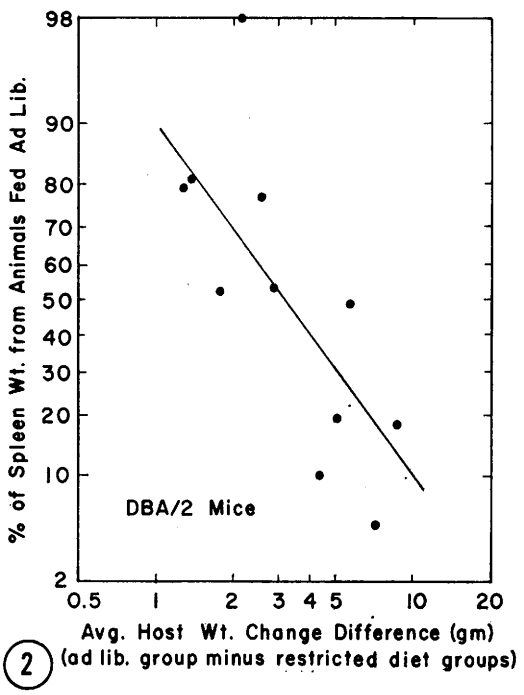
Exp	Food allowance (g/mouse /day)	Host body wt change* (g) Avg	Range	Difference (*ad lib* - restricted)	Spleen wt Avg (mg)	% of *ad lib* controls	% Splenomegaly inhibition per g host weight difference	Mortality	Virus titer† Spleen	Plasma
1	*Ad lib*	− .4	−8.0, +3.0		1301			0/10	10^{-3}	10^{-3}
	5.0	+ .6	−2.0, +2.0	−1.0	1699	130	—	0/10	10^{-1}	10^{-3}
	4.0	−2.6	−3.5, + .5	2.2	1384	106	—	0/10	10^{-1}	10^{-3}
	3.0	−2.2	−6.0, −1.0	1.8	678	52	27	0/10	10^{-1}	10^{-3}
	2.0	−4.8	−5.5, −4.0	4.4	129	10	20	0/10	10^{-3}	10^{-3}
2	*Ad lib*	+3.9	+3.0, +6.0		851			0/10	10^{-3}	10^{-3}
	5.0	+2.6	+2.0, +3.0	1.3	673	79	16	0/10	10^{-1}	10^{-3}
	4.0	+1.3	0.0, +4.0	2.6	653	77	9	0/10	10^{-3}	10^{-3}
	3.0	−1.9	−3.5, .0	5.8	408	48	9	0/10	10^{-1}	10^{-5}
	2.0	−4.8	−7.0, −3.0	8.7	157	18	9	0/10	10^{-1}	10^{-3}
3	*Ad lib*	+2.7	+ .5, +4.0		825			0/10	10^{-3}	10^{-3}
	5.0	+1.3	−1.0, +3.0	1.4	666	81	14	0/10	10^{-3}	10^{-3}
	4.0	− .2	−2.0, +2.5	2.9	440	53	16	0/10	10^{-3}	10^{-3}
	3.0	−2.4	−3.5, −1.0	5.1	155	19	16	0/10	10^{-3}	10^{-3}
	2.0	−4.5	−4.5, −1.0	7.2	40	5	13	0/10	10^{-3}	10^{-3}
							Avg = 12.4			
							S.E.‡ = ±2.4			

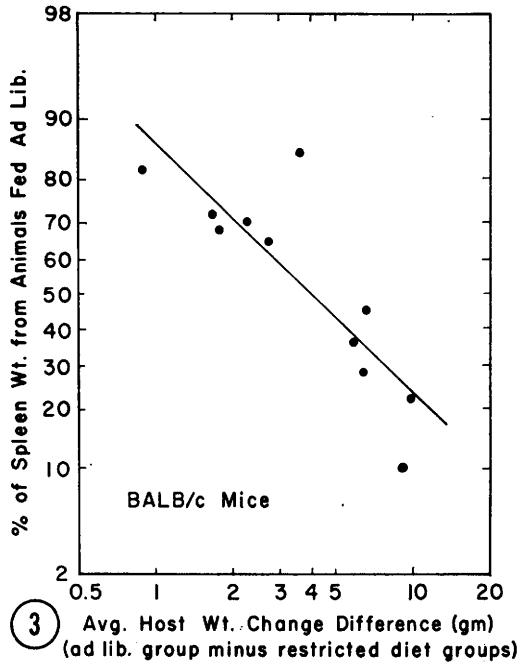
* Host body weight change = the difference in host weight on day of virus inoculation and day of sacrifice.

† Virus titer is expressed as the maximum dilution of Selas 03-filtered spleen suspension or plasma that will induce an average spleen weight of 400 mg in indicator Swiss mice.

‡ S.E. = Standard error as determined by *t* test.







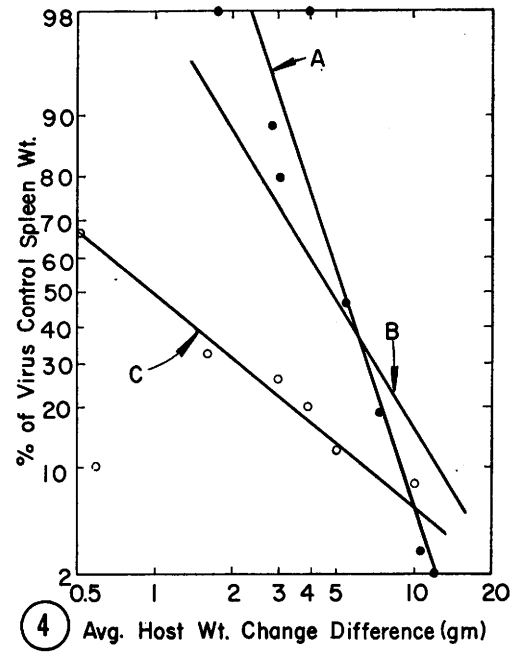


FIG. 1. Log probability plot of caloric restriction-induced host weight change difference *vs* splenomegaly inhibition. Friend leukemia virus in Swiss mice. Computer-analyzed line of best fit.

FIG. 2. Log probability plot of caloric restriction-induced host weight change difference *vs* splenomegaly inhibition. Friend leukemia virus in DBA/2 mice. Computer-analyzed line of best fit.

TABLE III. Effects of Caloric Restriction on Friend Leukemia Virus-Induced Splenomegaly and Infectivity of Spleens and Plasma in BALB/c Mice.

Exp	Food allowance (g/mouse /day)	Host body wt change* (g) Avg	Host body wt change* (g) Range	Difference (*ad lib* - restricted)	Spleen wt Avg (mg)	Spleen wt % of *ad lib* controls	% Splenomegaly inhibition per g host weight difference	Mortality	Virus titer† Spleen	Virus titer† Plasma
1	*Ad lib*	+1.6	- .5, +3.5		1038			0/10	10^{-3}	10^{-1}
	5.0	+ .7	-1.5, +3.0	.9	849	82	20	0/10	10^{-3}	10^{-3}
	4.0	- .7	-2.5, +2.0	2.3	732	70	13	0/10	10^{-5}	10^{-3}
	3.0	- 4.3	-6.5, - 1.5	5.9	372	36	11	0/10	10^{-5}	10^{-1}
	2.0	- 7.5	-9.5, - 5.5	9.1	101	10	10	4/10	10^{-3}	10^{-1}
2	*Ad lib*	+2.0	-2.5, +4.0		349			0/10	10^{-5}	10^{-5}
	5.0	+ .2	-2.3, +1.3	1.8	238	68	18	0/10	10^{-5}	10^{-3}
	4.0	- 1.7	-4.0, +1.0	3.7	296	85	4	0/10	10^{-5}	10^{-3}
	3.0	- 4.5	-7.0, - 1.5	6.5	97	28	11	2/10	10^{-5}	10^{-3}
	2.0	—		—	—	—	—	10/10	—	—
3	*Ad lib*	+2.5	.0, +5.0		161			0/10	10^{-2}	10^{-2}
	5.0	+ .8	.0, +3.0	1.7	116	72	16	0/10	10^{-2}	10^{-2}
	4.0	- .3	-2.0, +1.5	2.8	105	65	13	0/10	10^{-2}	10^{-2}
	3.0	- 4.1	-7.0, - 1.5	6.6	72	45	8	0/10	10^{-2}	10^{-2}
	2.0	- 7.3	-9.5, - 3.5	9.8	36	22	8	6/10	—	—
						Avg =	12.0			
						S.E.‡ =	±1.6			

* Host body weight change = the difference in host weight on day of virus inoculation and day of sacrifice.

† Virus titer is expressed as the maximum dilution of Selas 03-filtered spleen suspension or plasma that will induce an average spleen weight of 400 mg in indicator Swiss mice.

‡ S.E. = Standard error as determined by *t* test.

splenic tissue varied from 10^{-1} to 10^{-5} but loss of body or spleen weight had no apparent effect on these virus concentrations.

The results of studies with BALB/c mice were equivocal because marked splenomegaly could not be induced by FLV in 2 out of 3 experiments. These mice were inoculated with FLV at the same time as the DBA/2 mice previously described, so it is probable that sufficient virus was injected to induce splenomegaly. Relatively high virus titers were recovered from spleens and plasma of these animals, and the titers were unaffected by loss of body or spleen weight. Conflicting results have been reported concerning the relative susceptibility of BALB/c mice to FLV. Friend(5,8) reported these mice to be resistant to infection with this virus, while Fieldsteel(6,9) found this strain of mice to be equally, if not slightly more, susceptible than Swiss mice to the virus. All the BALB/c mice employed in the present studies were obtained from the same source but at different times.

In all experiments with BALB/c mice, loss of host weight also resulted in loss of spleen weight, with approximately a 12% splenomegaly inhibition per gram of host weight loss (Table III).

The caloric restriction-induced host weight change effects on splenomegaly are summarized in Fig. 1-3 for Swiss, DBA/2, and BALB/c mice. These data are presented as log probability plots of host weight difference *vs* splenomegaly inhibition, with a maximum likelihood line of best fit determined on a

FIG. 3. Log probability plot of caloric restriction-induced host weight change difference *vs* splenomegaly inhibition. Friend leukemia virus in BALB/c mice. Computer-analyzed line of best fit.

FIG. 4. A test of drug specificity. Comparison of caloric restriction-induced and drug-induced host weight change and associated splenomegaly inhibition in Swiss mice. Computer-analyzed line of best fit. A = 1-Methyl-1-nitrosourea-induced host weight change and splenomegaly inhibition; B = Caloric restriction-induced host weight change and splenomegaly inhibition (from Fig. 1); C = Porfiromycin-induced host weight change and splenomegaly inhibition.

TABLE IV. Comparison of Effect of Porfiromycin and 1-Methyl-1-Nitrosourea upon Friend Leukemia Virus Infections in Swiss Mice.

Compound	Dosage (mg/kg/day)*	Mortality	Avg host body wt change (g) C–T	Avg spleen wt (mg)	% of virus control spleen wt	Splenomegaly inhibition P†	% Reduction of‡ Plasma virus	% Reduction of‡ Spleen virus
Porfiromycin	8.0	0/10	5.5	48	5	<.01	62	84
	4.0	0/10	.6	96	10	<.01	60	80
	2.0	0/10	1.6	304	33	<.01	2	32
	1.0	0/10	– .4	393	43	<.05	30	19
	0 §	1/20		918				
1-Methyl-1-nitrosourea	24.0	8/10	12.3	18	2	<.01	0	0
	12.0	1/10	5.6	313	47	<.05	0	0
	6.0	1/10	4.2	931	140	—	0	0
	3.0	0/10	1.8	1304	196	—	0	0
	0 §	1/20		667				

* Treatment i.p. qd 1-9.
† P = Probability that splenomegaly inhibition was due to chance. Determined by *t* test.
‡ Virus in plasma and spleen determined by 21-day splenomegaly in indicator Swiss mice inoculated with the material.
§ Virus controls.

programmed Royal McBee LGP30 computer. This plot provides a standard of splenomegaly inhibition for each increment of host weight difference. Laster *et al*(10) indicated that such a plot would be helpful in evaluating the possibility that a given agent was providing no specific tumor inhibition beyond that associated with "nonlethal" toxicity. To aid in this evaluation, the host weight change difference resulting from therapy was plotted against tumor inhibition, and a comparison was made of this plot to the caloric restriction for the same strain of mice.

To determine if this simple test of drug specificity would apply to the Friend virus-induced splenomegaly system, 2 drugs, porfiromycin and 1-methyl-1-nitrosourea, which have been studied in chemotherapy experiments with this virus, were compared. Examples of the chemotherapy data for these compounds are shown in Table IV. In several studies both compounds significantly inhibited FLV-induced splenomegaly in Swiss mice, but further study showed that only porfiromycin reduced the amount of detectable virus in the plasma and spleens of these animals. The host weight change differences from 2 experiments with each compound were plotted against splenomegaly inhibition, and these plots were compared to the previously described caloric restriction-induced splenomegaly inhibition plot for Swiss mice. These plots are shown in Fig. 4. It can be seen that porfiromycin inhibits FLV-induced splenomegaly, and this inhibition is separate from the effects of host inanition due to caloric restriction. The effects of 1-methyl-1-nitrosourea, however, could not be dissociated from splenomegaly inhibition caused by caloric restriction-induced host weight change.

Summary. Caloric restriction studies in Swiss, DBA/2, and BALB/c mice infected with Friend leukemia virus (FLV) have indicated that loss of host weight can markedly affect the extent of virus-induced splenomegaly, but does not noticeably affect the titer of recoverable virus in plasma or spleens from these animals. It was concluded that host weight change is important and should be considered when using FLV-induced splenomegaly in chemotherapy trials. The effect on FLV-induced splenomegaly of 2 compounds, porfiromycin and 1-methyl-1-nitrosourea, were evaluated in the light of these findings.

1. Tannenbaum, A., Ann. N. Y. Acad. Sci., 1947, v49, 5.
2. Sugiura, K., Gann, 1959, v50, 251.
3. Mirand, E. A., Back, N., Prentice, T. C., Ambrus, J. L., Grace, J. T., Jr., Proc. Soc. Exp. Biol and Med., 1961, v108, 360.
4. Sidwell, R. W., Dixon, G. J., Sellers, S. M., Antimicrob. Agents & Chemother., 1964, p767.
5. Friend, C., J. Exp. Med., 1957, v105, 307.
6. Fieldsteel, A. H., Dawson, P. J., Bostick, W. L.,

Proc. Soc. Exp. Biol. and Med., 1961, v108, 826.
7. Rowe, W., Brodsky, P., J. Nat. Cancer Inst., 1959, v23, 1239.
8. Friend, C., Perspectives in Virology, M. Pollard, Ed., John Wiley and Sons, New York, 1959, 231.
9. Fieldsteel, A. H., Dawson, P. J., Bostick, W. L., Cancer Research, 1963, v23, 355.
10. Laster, W. R., Jr., Schabel, F. M., Jr., Skipper, H. E., Wilcox, W. S., Thomson, J. R., ibid., 1961, v21, 895.

Received May 6, 1965. P.S.E.B.M., 1965, v119.

Cardiac Phosphorylase in Different Species and after Reserpine Administration.* (30399)

NARANJAN SINGH DHALLA,† NIELS HAUGAARD AND MARILYN E. HESS‡
Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Several species of animals have been used in studies of cardiac phosphorylase activity (1), and there appears to be some difference in enzyme activity from one species to another. In addition, differences in activity of phosphorylase within the various layers of the heart have been demonstrated. For example, Jedeikin(2) has shown that phosphorylase activity increased progressively from the epicardium to the endocardium in dog and rabbit hearts.

The present investigation is concerned with the phosphorylase activity in hearts from 3 species, *i.e.*, rabbit, rat and frog. The studies also include measurements of the enzyme in the ventricles and atria from the 3 species as well as studies of the effect of reserpine administration on ventricular and atrial phosphorylase.

Methods. The animals were male New Zealand white rabbits (1.8-2.0 kg), male albino rats of the Wistar strain (200-250 g) and male and female frogs (*Rana pipiens,* 30-50 g).

Rats and rabbits were killed by a blow on the head and frogs were pithed. After opening the thorax, the heart was quickly removed, cooled in an empty beaker kept in crushed ice. Portions of tissue were dissected as described below, weighed on a torsion balance and extracts prepared immediately by grinding the tissue in ice-cold mortars containing a solution of 0.001 M $EDTANa_2$-0.02 M NaF (3 ml/100 mg tissue wet weight). In the experiments with rabbit hearts and with some of the rat hearts, the left and right atria and the middle portions of the left and right ventricles were used for phosphorylase assay; in other experiments with rat hearts (Table III), enzyme activity was determined in the whole atria and the apical portions of the ventricle. When frog hearts were used, the atrium was separated from the ventricles and each of these portions extracted for enzyme assay. When reserpine was administered, the drug was given in a single intraperitoneal injection, unless otherwise indicated, at doses of 2.5 or 5.0 mg/kg. The animals were sacrificed at stated intervals following drug administration.

Phosphorylase determination. Heart extracts were centrifuged in the cold room and the supernatant fluid was further diluted 3 times with EDTA-NaF solution so that the final concentration of tissue was 6.54 mg/ml. The extracts were kept in an ice bath and analyzed for enzyme activity within 4 hours of their preparation.

Phosphorylase activity was determined in the direction of glycogen synthesis ($\text{glucose-1-P} + \text{glycogen}_{(n)} = \text{Pi} + \text{glycogen}_{(n+1)}$) according to the method of Cori and Illingworth(3) as follows: 0.5 ml extract (3.27 μg tissue) was incubated at 30° for 5 minutes with 0.5 ml substrate mixture and the forma-

* Supported by grants from Am. Heart Assn. and Nat. Heart Inst. (HE-01813).

† The data presented in this paper are taken from a thesis submitted by N. S. Dhalla in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology.

‡ This work was done during the tenure of an Established Investigatorship of Am. Heart Assn.