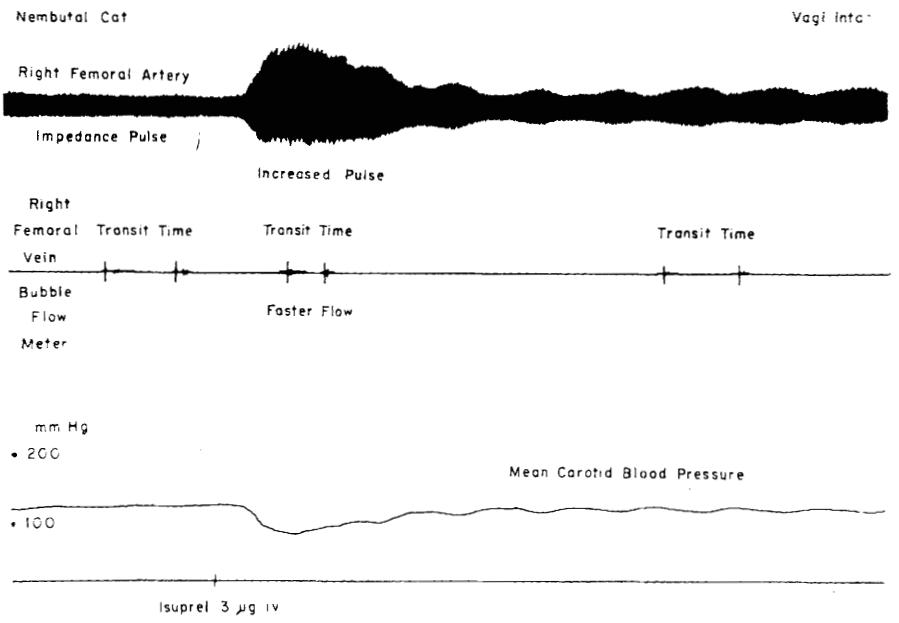

FIG. 3.
Direct Flow—Volume Pulse Comparison, Dilator Effect.

be demonstrated in this manner and indications of relative changes in blood flow can be obtained. With these methods hemodynamic responses to pharmacologic agents can be studied. The initial responses and subsequent adjustments can be followed.

Received August 21, 1950. P.S.E.B.M., 1950, v75.

Influence of Fasting and Adrenalectomy on Normal and Malignant Lymphoid Tissue Composition.* (18275)

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Among considerations of interest in the study of a neoplastic tissue is its responsiveness to specific physiologic stimuli known to have characteristic influences on homologous normal tissue. The well-known involutional response of lymphoid tissue to pituitary-adrenal cortical secretions, and, through their mediation, to a variety of stresses(1), has prompted investigations of the sensitivity of lymphoid tumors to these influences. Such studies have already indicated that in laboratory animals(2,3), and in humans(4), strik-

* This investigation was aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

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ing regression of lymphoid tumors occurs during treatment with pituitary adrenocorticotrophic hormone or adrenal cortical steroids.

Previous studies have shown that the considerable loss in weight and nitrogen content of the pooled lymphoid tissue (spleen, lymph nodes and thymus) which occurs during a 48-hour fast in CBA mice is dependent upon adrenal secretions and fails to occur in the adrenalectomized, fasted animal(5). The availability in mice of the same strain of a rapidly growing transplantable lymphosarcoma which metastasizes only late and consists of cells morphologically similar to normal lymphocytes(6), permitted the extension of such observations to tumor-bearing animals. The present report deals with a study of the weight and composition of normal lymphoid structures and of the tumor as these values were influenced by a 48-hour fast in intact and adrenalectomized mice. Data describing changes in the liver weight and composition of fasted, lymphosarcoma-bearing mice have been presented previously (7).

Methods. Male mice of the CBA strain (Strong) weighing 20 to 25 g and 8 to 9 weeks of age were used. All animals were fed the standard laboratory diet of Rockland chow and, during the period of fasting, were housed in individual metabolism cages with free access to water. Fed mice used as controls for fasting experiments were similarly kept in metabolism cages for the duration of the fasting period, to eliminate the considerable difference in activity which larger cages permit. Mice prepared for fasting experiments were inoculated with a small fragment of lymphosarcoma(6) maintained in the same strain. At varying intervals thereafter, groups of 5 to 7 mice were subjected to a 48-hour fast, while comparable control groups were fed the laboratory diet, as noted above. At the termination of the fasting period, each mouse of both groups was autopsied. Tissues removed routinely for weight and analysis were liver, spleen, tumor, pooled thymus and mesenteric lymph nodes, and a sample of the muscle (gastrocnemius). The entire liver and tumor‡ were weighed to the nearest 5 mg on a pharmaceutical torsion balance; the spleen, thymus-nodes and muscle sample were weighed to the nearest mg either on a Roller-Smith torsion balance or on an analytical balance in connection with water analysis. Aliquots of each tissue were taken for nitrogen analysis by the micro-Kjeldahl procedure(8). Water content was determined by drying small aliquots of tissue overnight in an oven at 110°C. Tissue protein was approximated by multiplying percent nitrogen by the factor 6.25; tissue lipid was then calculated by difference from the values for percent protein and percent water(9). In some instances (Experiments V, X, XI in Tables I and II), groups of fed mice were autopsied at the beginning of a fasting period to provide initial data with which fasted and fed groups autopsied at the end of a 48-hour fast could be compared. Bilateral adrenalectomy was performed in tumor-bearing mice under ether anesthesia, and the animals maintained continuously thereafter on 1% saline drinking water. Fasting experiments were initiated in 2 such groups of mice 24 hours (Experiment X) and 48 hours (Experiment XI) following operation.

Results. Body weight. As is indicated by the weight data in Table I, fed mice in which the tumor is growing experience a progressive gradual increase in total body weight which is only partly accounted for by the increase in mass of the tumor itself. Since

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‡ In all animals except those of Exp. IX (Tables I and II), the tumor was well-localized and satisfactorily complete and clean removal was possible.

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TABLE I.* Effect of a 48-hr Fast on Tumor and Lymphoid Tissue Weight in Unoperated and Adrenalectomized Lymphosarcoma-bearing Male Mice. Each experiment represents paired groups of mice autopsied at the same time, with the exception of Experiments V, X, and XI, in which an initial fed group was autopsied at the beginning of the fasting period.

Exp.	Group	No. of mice	Age (wk)	Tumor age (days)	Body wt (g)	Wt in mg		
						Tumor	Thymus-nodes	Spleen
				Normal mice				
I	Fed	7	8.1	—	25.3 ± .6	--	103 ± 4	74 ± 2
II	Fed	5	8.0	—	21.6 ± .3	—	92 ± 5	85 ± 2
	Fasted	5	8.0	—	17.6 ± .7	—	68 ± 5	51 ± 4
				Tumor-bearing mice				
III	Fed	5	8.3	2	20.0 ± .9	<10	78 ± 3	74 ± 3
	Fasted	5	8.3	2	15.8 ± .8	<10	53 ± 3	41 ± 3
IV	Fed	5	8.3	6	20.9 ± .9	<10	68 ± 6	127 ± 14
	Fasted	5	8.3	6	15.9 ± .8	<10	39 ± 4	59 ± 6
V	Fed	5	8.3	4	22.4 ± 1.2	<10	70 ± 7	110 ± 11
	Fed	4	8.6	6	22.1 ± .7	279 ± 40	65 ± 6	113 ± 13
	Fasted	5	8.6	6	16.8 ± .7	15 ± 10	50 ± 7	55 ± 3
VI	Fed	6	8.7	7	24.5 ± .5	530 ± 28	78 ± 4	115 ± 9
	Fasted	5	8.7	7	16.4 ± .3	80 ± 31	49 ± 6	55 ± 4
VII	Fed	6	8.3	9	24.0 ± .8	177 ± 30	105 ± 6	77 ± 3
	Fasted	5	8.3	9	19.0 ± .7	117 ± 35	54 ± 6	54 ± 5
VIII	Fed	6	8.1	11	26.2 ± .8	1577 ± 233	80 ± 6	124 ± 7
	Fasted	6	8.1	11	19.2 ± .7	428 ± 112	45 ± 2	65 ± 3
IX	Fed	3	8.4	15	30.2	3580	103†	163†
	Fasted	2	8.4	15	21.1	2340	38	79
				Adrenalectomized, tumor-bearing mice				
X§	Fed	5	8.1	7	20.5 ± .8	<10	81 ± 8	143 ± 20
	Fed	5	8.4	9	21.2 ± .7	244 ± 108	92 ± 3	142 ± 7
	Fasted	7	8.4	9	21.2 ± 1.0	150 ± 60	82 ± 6	137 ± 11
XI‖	Fed	5	9.6	11	22.6 ± 1.0	527 ± 60	95 ± 6	163 ± 9
	Fed	4	9.9	13	22.8 ± .9	1060 ± 221‡	89 ± 6	122 ± 6
	Fasted	6	9.9	13	20.9 ± .5	590 ± 102	80 ± 7	108 ± 11
	Fed¶	4	9.9	13	31.2 ± .7	1980 ± 209	106 ± 16	185 ± 19

* Values shown are means and stand. errors. Stand. errors are not shown for groups of less than 4 individuals.

† Grossly enlarged and thought to be tumor infiltrated.

‡ Group of 7 mice.

§ Adrenalectomized on 6th day following tumor implantation.

‖ Adrenalectomized on 9th day following tumor implantation.

¶ Non-adrenalectomized controls given tumor on same day as remaining mice in Exp. XI.

no significant increase in the water content of muscle (Table II) occurs during the period of tumor growth, the possibility of progressive generalized hydration seems contraindicated. The percent loss of body weight during a 48-hour fast, as calculated by comparing fed and fasted tumor-bearing animals at the termination of the fasting period, similarly increases with the size which the tumor has attained by the beginning of the fast. Fasted mice free of tumor lose, under these conditions, approximately 20% of their body weight, while a similar fast begun when the tumor has been present from 7 to 13 days results in a difference of 27 to 30% between the weights of fasted and fed animals. At least part of this increment of weight loss is accounted for by the inhibition of growth of the tumor itself, as described below. As previously noted(9,10), the adrenalectomized mouse fails to respond to a 48-hour fast with a loss of body weight as great as that found in the intact fasted animal. It is evident from the data of Table I that the presence of a tumor does not alter this inhibition of body weight loss by adrenalectomy. In the case of Experiment X, the surprising failure to demonstrate any mean weight difference between fasted and fed animals was due to

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TABLE II.* Percent Composition of Water, Protein and Lipid in Tumor, Lymphoid Tissue and Muscle of Fed and Fasted Unoperated and Adrenalectomized Tumor-bearing Mice. Experiment numbers correspond to those in Table I.

Exp.	Group & No.	Tumor age (days)	Percent of each component: Tumor Water	Tumor Protein	Tumor Lipid	Thymus nodes Water	Thymus nodes Protein	Thymus nodes Lipid	Spleen Water	Spleen Protein	Spleen Lipid	Muscle Water	Muscle Protein	Muscle Lipid
						Normal mice								
I	Fed(7)	—	—	—	—	74.9	15.5	9.7 ± .9	78.3	17.6	4.4 ± .4	75.3	20.7	3.9 ± .6
						Tumor-bearing mice								
IV	Fed(5)	6	—	—	—	72.0	12.1	15.9 ± 3.4	79.0	16.6	4.8 ± .3	75.2	19.4	5.3 ± .4
	Fasted(5)	6				79.2	15.3	5.5 ± .8	78.8	16.8	4.4 ± .4	76.1	18.3	5.5 ± .5
VI	Fed(6)	7	73.7	10.2	16.1 ± 1.1	77.3	12.4	9.2 ± 1.1	79.1	16.8	4.1 ± .2	77.7	19.3	2.9 ± .6
	Fasted(5)	7	68.1	11.0	20.9 ± 3.5	80.3	14.2	5.7 ± .5	79.4	17.0	3.6 ± .3	73.1	19.5	7.4 ± 1.1
VII	Fed(6)	9	73.4	12.9	10.3	77.6	14.7	7.9 ± .8	79.1	17.7	3.5 ± .2	74.3	19.7	6.6 ± 1.4
	Fasted(6)	9	73.2	14.3	9.4	80.5	15.0	4.5 ± .4	78.4	18.5	3.1 ± .2	75.4	21.9	1.9 ± .5
VIII	Fed(6)	11	79.3	12.7	7.9 ± 1.8	77.5	14.3	7.8 ± 1.1	79.0	16.4	4.7 ± .2	75.7	19.2	5.5 ± .9
	Fasted(6)	11	80.9	13.7	5.7 ± .4	79.4	15.0	5.5 ± .6	77.1	17.7	5.6 ± 1.2	75.7	21.5	3.2 ± .6
IX	Fed(3)	15	81.5	15.6	2.9	80.5	13.2	6.3	79.8	16.2	4.9	75.9	19.5	2.9
	Fasted(2)	15	81.9	14.9	3.1	79.8	15.1	5.2	78.6	17.4	4.0	77.5	18.9	3.5
						Adrenalectomized, tumor-bearing mice								
X	Fed(5)	7	—	—	—	76.1	14.0	9.9 ± .6	78.9	16.9	4.1 ± .2	76.3	19.8	3.9 ± .5
	Fed(5)	9	74.9	17.2	11.8 ± 4.2	75.4	13.9	10.4 ± 1.9	79.1	16.3	4.6 ± .2	74.5	19.8	5.7 ± .2
	Fasted(7)	9	78.2	14.2	10.1 ± 2.7	78.2	12.6	9.1 ± .4	79.2	15.6	5.2 ± .4	76.5	18.9	4.6 ± .5
XI	Fed(5)	11	75.1	12.7	12.5 ± 3.4	78.4	13.7	7.9 ± .8	79.3	16.2	4.5 ± .2	75.9	19.0	5.1 ± 1.1
	Fed(4)	13	77.5	14.3	8.2	78.7	13.9	7.4 ± 1.3	78.8	16.6	4.6 ± .5	76.8	18.2	5.1 ± .9
	Fasted(6)	13	79.9	14.9	5.4 ± 1.0	78.3	14.9	6.9 ± 1.9	79.1	15.0	5.8 ± .7	76.5	18.8	4.8 ± .6

* Values shown are means and standard errors. Standard errors have been calculated only for the lipid percent, except where groups contain less than 4 mice.

the presence, in several of the fasted mice, of distinct ascites unassociated with any intraperitoneal extension of the tumor.

Tumor weight and composition. The most striking feature of the tumor data is the consistent observation that the mean tumor weight in fasted mice was significantly lower than in fed controls. That this weight differential resulted not from an actual loss in weight of the tumors in fasted animals, but rather from a greatly reduced rate of tumor growth, is demonstrated by Experiment V, in which data derived by comparison of tumor weights in the fasted mice with those of a group of tumor-bearing animals autopsied at the beginning of the fasting period, reveal minimal growth of the tumor during the fast. The failure of adrenalectomy to inhibit this retardation of tumor growth is in contrast to the previously observed inhibitory effect of adrenalectomy on the fasting involution of normal lymphoid tissue. As in the unoperated mice, the difference between mean tumor weight in fasted and fed adrenalectomized mice also appeared to result from a retardation of the rate of tumor growth rather than from an absolute reduction in the initial size of the tumor. No specific effect of adrenalectomy upon the weight or growth rate of the tumor in fed animals can be established from these data. It is of interest to note, however, that in a small group of fed, non-operated controls (Table I, Exp. XI), the mean tumor weight 13 days following implantation was twice the mean weight of tumor in fed, adrenalectomized mice of the same group. This retardation of growth rate cannot be attributed to a specific inhibitory effect of adrenalectomy, since the inanition succeeding adrenalectomy might suffice in itself to account for the data. It is also of interest that no consistently distinct changes in the composition of the tumor result from a 48-hour fast (Table II), in contrast to the depletion of lipid induced in thymus and mesenteric lymph nodes by a similar fast (Table II and below). Although the actual values at each interval are not reliable (as a result of considerable individual variation both in tumor weight and composition), data concerning the lipid composition of the tumor (Table II) during its growth in fed mice, indicate a consistently progressive depletion of lipid between the first and second week following implantation.

Thymus, lymph nodes and spleen. The involution of normal lymphoid structures during the course of a 48-hour fast in normal mice, and the dependence of this phenomenon on the presence of the adrenals(9,10) is also seen in the tumor-bearing mouse, although the magnitude of the involutional weight reduction on fasting is not uniformly as great as that established previously. The data of Table I indicate, in addition, a reduction in thymus-mesenteric node weight and an increase in spleen weight in fed tumor-bearing mice as compared with fed tumor-free controls. As is true of the tumor, a progressive depletion of lipid occurs in the thymus and mesenteric nodes of fed tumor-bearing mice as the tumor attains increasing age and mass (Table II). The remarkably high value of 15.9% lipid calculated for the thymus and nodes of fed mice in Experiment IV cannot be regarded as reliable, however, because of the high degree of individual variation in the analyses of this group. In contrast to the failure to demonstrate consistent depletion of lipid in the tumors of fasted animals, the characteristic depletion in lipid content in the thymus and mesenteric nodes induced by fasting(10), is maintained in the presence of the tumor. Adrenalectomized tumor-bearing mice, similarly, fail to mobilize fat from the thymus and mesenteric nodes during a fast to the same extent as is noted in fasted, unoperated controls.

Discussion. The striking retardation in the growth of a mouse lymphosarcoma resulting from a 48-hour fast indicates the dependence of this rapidly growing tissue on an undiminished exogenous source of energy and cell constituents. The persistence of its growth during the fast, albeit at a greatly reduced rate, and at the expense of the already depleted tissues of the fasting host, however, is consistent with the well-known autonomy of growth of malignant tumors. Unlike the physiologic involution of lymphoid

tissue induced by the stress of a fasting period, this reduction in growth rate of the tumor is not apparently dependent on the maintenance of adrenal cortical secretions, an observation which demonstrates a distinct difference in behavior between the tumor and its morphologically homologous normal counterpart. However, other observations (2,3,4) have indicated that under some conditions malignant lymphoid tissue responds as does normal lymphoid tissue to certain endocrine stimuli. The persistence of the fasting involution of uninvolved lymphoid structures in lymphosarcoma-bearing mice, and its inhibition by adrenalectomy, serves to emphasize the difference in behavior between malignant and normal lymphoid tissue with respect to pituitary-adrenal cortical influence.

That the reduction in the mass of pooled thymus and mesenteric lymph nodes noted in fed, tumor-bearing mice may be adrenal-mediated is suggested by its failure to occur in the adrenalectomized animal, in which, indeed, the spleen attains a mass even greater than that noted in the presence of the tumor alone. It seems equally possible, however, that the presence of the tumor and adrenalectomy represent 2 independent influences on thymus-node mass with opposing effects, the resultant of which is exhibited in the preservation of normal lymphoid tissue weight.

Further data of interest relate to the relatively high lipid content of the tumor (and possibly of the pooled thymuses and mesenteric nodes of fed tumor-bearing animals), and the progressive decline of lipid concentration in these structures as the tumor attains increasing size in the fed animal. Whether this observation indicates a specific and important role of the tumor in the metabolism of lipid, either in the tumor tissue or in other tissues as well, and what its relation may be to the influence of the tumor in the production of fatty livers in fasted mice (7), is unknown at the present time.

Summary. Weight measurements and composition analyses of a transplantable lymphosarcoma and several normal lymphoid tissues in fasted, tumor-bearing CBA mice indicate that a 48-hour fast results in a marked retardation of tumor growth rate without completely inhibiting absolute growth. This tumor growth differential in fasted and fed mice is not dependent on the presence of the adrenals. On the other hand, uninvolved, non-malignant lymphoid structures in tumor-bearing animals show qualitatively the same involutional effect of fasting and the same dependence of this phenomenon on the presence of the adrenals as has been reported for tumor-free animals. Thus, malignant lymphoid cells (transplantable lymphosarcoma) in fasted mice exhibit a striking independence of the pituitary-adrenal cortical control which regulates the fasting involution of non-malignant lymphoid tissue. A considerably greater weight of the spleen was seen in fed, tumor inoculated mice, as compared to control animals. This splenic enlargement was apparent even at an early time following tumor inoculation when no tumor mass was palpable. The transplantable lymphosarcoma is relatively high in lipid content early in its development, but the percentage of lipid steadily declines as tumor size increases.

Mrs. Rita Huang contributed valuable assistance with the analytical and operative procedures.

Received October 10, 1950. P.S.E.B.M., 1950, v75.