TABLE III. Effect of NAF on Agglutination of Chicken and Human Red Blood Cells by Mumps and Influenza Viruses.

Virus*	Cells	Final dilution of NAF 1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Saline
Mumps	C	0	0	0	±	2	3	3	2	3
	H	0	0	0	0	±	1	2	3	4
Lee	C	0	±	3	4	4	4	4	4	4
	H	4	4	4	4	4	4	4	4	4
PR-8	C	0	0	1	2	3	3	3	3	3
	H	0	4	4	4	4	4	4	4	4
Saline Controls	C	0								0
	H	0								0

* 2 hemagglutinating units present in each instance.

with varying amounts of NAF and tested with chicken and human erythrocyte suspensions. Although the results with mumps virus are most striking, they are not essentially different from those obtained with PR-8 and Lee viruses. However, in contrast to the findings with mumps virus, with the influenza viruses the effect is more obvious with chicken cells than with human cells.

Discussion. The results of these experiments suggest that with mumps virus the equation introduced by Hardy and Horsfall (3) to explain the virus-inhibitor reaction:

$$\underset{\text{(Virus)}}{V} + \underset{\text{(Inhibitor)}}{I} \rightleftharpoons \underset{\text{(Virus—Inhibitor combination)}}{VI}$$

is influenced in the presence of human cells to move in the direction of combined virus (VI). Consequently, agglutination which depends upon the presence of free virus may not be apparent until the fluid is diluted beyond the zone of effective activity of the inhibitor. Since this same fluid before dilution may show clear agglutination of chicken cells, it would seem that in the presence of chicken cells the equation is influenced in the reverse direction (in the direction of free virus). With PR-8 and Lee viruses the same type of activity can be presumed to take place, with the exception that in those systems the chicken cells furnish something which influences the equation in the direction of combined virus.

Summary. It is shown that the inhibitor present in allantoic fluid for hemagglutination by and absorption of mumps virus is more active when human erythrocytes rather than when chicken red blood cells are used. It appears that the species of erythrocyte present influences the reaction between mumps virus and inhibitor in the direction of more or less combined (non-hemagglutinating) virus. A similar influence, though to a less striking degree, is also shown for the red blood cell in the influenza virus-inhibitor reaction.

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Development of Fatty Livers in Fasted Male Mice Bearing a Transplantable Lymphosarcoma.* (18171)

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A variety of experimental conditions including dietary alterations, endocrine treatment, and the administration of toxic substances, are known to result in the development of fatty livers in laboratory animals (1). During the course of an investigation

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of the influence of a rapidly growing transplantable lymphosarcoma on the metabolism of the host, accumulation of lipid has been noted in the livers of tumor-bearing male mice fasted for 48 hours. By contrast, fatty livers did not appear in similar tumor-bearing mice fed the normal laboratory diet of Rockland chow, or in normal male mice fasted for 48 hours under the same conditions.

Methods. Male mice of the CBA strain (Strong) weighing 20 to 25 g and 8 to 9 weeks of age were used. Sex, age and strain specification is of the utmost importance, since it is well known that such variables influence the susceptibility of animals to the development of fatty livers on fasting alone. Three-month-old albino male mice under specified conditions have been reported(2) to develop fatty livers after a 24- to 48-hour fast, although the relation of maximum liver fat to day of fasting could be modified by changes in the environmental temperature. Similarly it has been noted in connection with the observations to be reported that older CBA male mice at about 16 weeks of age also develop fatty livers as the result of a 48-hour fast alone. For these reasons, it must be emphasized that at the termination of a 48-hour fast under the conditions of the present observations, the livers of normal CBA male mice 7 to 10 weeks of age show only a slightly increased percentage of lipid, and (because of considerable loss in liver weight), a prominent reduction of total lipid, expressed both in absolute quantity and relative to body weight(3).

Mice prepared for fasting experiments were inoculated subcutaneously with a small fragment of a transplantable lymphosarcoma(4). At varying intervals thereafter, groups of 5 to 7 animals were subjected to a 48-hour fast with free access to water, while control groups received the laboratory diet. At the end of the fasting period each mouse in both groups was autopsied and aliquots of liver tissue analyzed for water content by drying at 110°C, and for nitrogen concentration by the micro-Kjeldahl procedure(5). From these data, liver lipid concentration was calculated by difference, a method whose validity has been established by comparison with direct chemical analysis for total lipid(3). Mice of similar age‡ were bilaterally adrenalectomized 6 to 9 days following tumor inoculation and were subjected to a 48-hour fast beginning 24 to 48 hours following operation. All adrenalectomized animals were maintained continuously on 1% saline as drinking fluid. All experimental mice, either fasted or fed, were kept in individual metabolism cages for the duration of the 48-hour fasting period. The environmental temperature was not held constant but was generally between 20 and 25°C.

Results. The livers of fasted tumor-bearing mice in almost all groups were visibly fatty on gross inspection. Under these conditions, mice free of tumor did not develop fatty livers as the result of fasting alone, as has been previously demonstrated(3). Similarly, the presence of the tumor in fed mice, caged under identical conditions of temperature and opportunity for activity, never resulted in the development of fatty livers. The opposite trend, in fact, is indicated by the data shown in Table I, *i.e.*, a tendency for the livers of tumor-bearing fed mice to contain both a slightly lower concentration and total content of lipid, as compared with fed mice free of tumor.

As is evident from the data presented, a 48-hour fast resulted in distinctly fatty livers at varying intervals after tumor implantation, and without quantitative correlation with tumor size. This phenomenon did not occur either very early (2 days) following tumor inoculation, or very late in the development of the tumor, at a time when the mice appeared moribund. It is notable, however, in the data of Experiment 4 that markedly fatty

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‡ The mice of Experiment XI were 9 to 10 weeks of age at the time of autopsy.

TABLE I.* Effect of a 48-hour Fast on Concentration of Liver Lipid in Fed and Fasted Unoperated and Adrenalectomized Lymphosarcoma-bearing Male Mice. Each experiment represents paired groups of mice autopsied at the same time, with the exception of Exp. V, X, and XI, in which an initial fed group was autopsied at the beginning of a 48-hour fasting period.

Exp.	Group	No. of mice	Tumor age (days)	Tumor wt (mg)	Body wt (g)	Liver wt (mg)	% liver lipid
				Normal mice			
I	Fed	7	—	—	25.3 ± .6	1800 ± 76	10.4 ± .9
II	Fed	5	—	—	21.6 ± .3	1330 ± 42	10.3 ± .4
	Fasted	5	—	—	17.6 ± .7	960 ± 33	10.8 ± 1.3
				Tumor-bearing mice			
III	Fed	5	2	<10	20.0 ± .9	1250 ± 33	10.0 ± .8
	Fasted	5	2	<10	15.8 ± .8	750 ± 47	10.0 ± 1.3
IV	Fed	5	6	<10	20.9 ± .9	1260 ± 25	9.6 ± 1.0
	Fasted	5	6	<10	15.9 ± .8	1090 ± 72	18.8 ± 1.5
V	Fed	5	4	<10	22.4 ± 1.2	1370 ± 70	9.4 ± .2
	Fed	4	6	279 ± 40	22.1 ± .7	1350 ± 66	10.6 ± .3
	Fasted	5	6	15 ± 10	16.8 ± .7	1270 ± 57	27.1 ± .9
VI	Fed	6	7	530 ± 28	24.5 ± .5	1650 ± 56	7.6 ± 1.0
	Fasted	5	7	80 ± 31	16.4 ± .3	1120 ± 39	16.0 ± .7
VII	Fed	6	9	177 ± 30	24.0 ± .8	1420 ± 56	7.3 ± .4
	Fasted	6	9	117 ± 35	19.0 ± .7	1180 ± 26	11.8 ± 1.6
VIII	Fed	6	11	1580 ± 233	26.2 ± .8	1570 ± 58	8.5 ± .7
	Fasted	6	11	428 ± 112	19.2 ± .7	1350 ± 75	13.9 ± .3
IX	Fed	3	15	3580	30.2	1630	9.0
	Fasted	2	15	2340	21.1	900	6.1
				Adrenalectomized tumor-bearing mice			
X‡	Fed	5	7	<10	20.5 ± .8	1240 ± 99	7.4 ± .9
	Fed	5	9	244 ± 108	21.2 ± .7	1170 ± 9	8.2 ± .3
	Fasted	7	9	150 ± 60	21.2 ± 1.0	1080 ± 32	15.1 ± .9
XI§	Fed	5	11	527 ± 60	22.6 ± 1.0	1270 ± 107	8.9 ± 1.2
	Fed	4	13	1060 ± 221†	22.8 ± .9	1300 ± 74	8.9‖
	Fasted	6	13	590 ± 102	20.9 ± .5	1220 ± 67	16.9 ± 2.9

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

† Represents group of 7 mice.

‡ Adrenalectomized on the 6th day following tumor implantation.

§ Adrenalectomized on the 9th day following tumor implantation.

‖ Mean of 3 values.

livers were seen in fasted mice in the presence of tumors of minute size, not grossly palpable.

In 2 instances (see Table) experiments similar to those above were carried out with lymphosarcoma-bearing fasted mice which had been bilaterally adrenalectomized 1 or 2 days prior to the beginning of the fasting period. In such animals no inhibition of the development of fatty livers on fasting appeared to result from the absence of the adrenals.

Discussion. These observations, describing the appearance of fatty livers in fasted mice carrying a transplantable lymphosarcoma, throw no light on the mechanism of its production. Indeed, the specificity of this phenomenon for tumors of specific cellular type, or for stresses of more generalized character, requires further investigation. It is conceivable that the presence of a rapidly growing tumor, like exposure to cold(6), represents an additional demand for calories beyond that required by a fasted animal, and that the accumulation of excessive liver lipid may result from the increased mobilization of depot fat necessary to meet this additional caloric demand. That the presence even of a minute tumor, seemingly too small to create a significant caloric demand by virtue of its own energy metabolism, is sufficient to determine the development of a fatty liver in the fasted host, suggests the alternate possibility of a more specific, perhaps humoral, influence on

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fat metabolism. Furthermore, the persistence of lipid accumulation in the absence of the adrenals would seem to distinguish this type of fatty liver from those described in previous studies(6,7) which implicated the adrenal cortex in the mobilization of fat to the liver under circumstances of stress. In this connection, it may be of significance that one of the few experimental procedures which result in fatty livers in fasted male mice and is similarly effective even in the absence of the adrenals, is the administration of purified growth hormone(3).

Summary. The development of fatty livers has been noted in 8-week-old fasted CBA male mice bearing a transplantable lymphosarcoma of varying age. Similar mice free of the tumor fail to develop fatty livers following a 48-hour fast under comparable conditions, nor does the growth of the tumor in fed mice result in fatty livers. Adrenalectomy has no inhibitory effect on the development of a fatty liver under these conditions.

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Urinary Excretion of Certain Amino Acids During ACTH and Cortisone Treatment of Rheumatoid Arthritis.* (18172)

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In a previous publication from this laboratory(1) it was shown that the urinary excretion and plasma levels of free histidine were increased in patients with rheumatoid arthritis treated with ACTH (adrenocorticotropic hormone) and Cortisone (17-hydroxy-11-dehydro corticosterone). Urinary excretion and plasma values of 17 free and bound amino acids are being studied in conjunction with a clinical study to be reported elsewhere(2). The present paper summarizes the urinary excretion of free threonine, lysine, tyrosine, and arginine in 41 patients before and after treatment.

Experimental. Thirty-one patients (12 ♂ and 19 ♀) were treated with ACTH and 10 patients (5 ♂ and 5 ♀) with Cortisone. All patients suffered from active rheumatoid arthritis and had been under observation for at least 6 months. They were hospitalized and controlled metabolically during the entire period of investigation. ACTH, given intramuscularly, in variable daily dosage (20 to 160 mg) was continued for a period of 8 to 17 days. Cortisone was given intramuscularly usually in an initial dose of 300 mg followed by 100 mg on successive days for about 10 days. All patients were not represented in determinations of free arginine and tyrosine. Various patients, receiving ACTH, were pretreated and treated simultaneously with one or more of the following in an effort to enhance or prolong the beneficial effect of ACTH: 6 were treated with thyroid and ascorbic acid, 4 with ascorbic acid alone, 6 with ATP (adenosine triphos-

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