in the majority it was not present until after day 40. Once antibody appeared in the ascites fluid it continued to be present in subsequent collections up to day 67, when the experiments were terminated.

The advantages of this method of obtaining antibody are obvious. The method is simple, uses an inexpensive animal from which fluid can be obtained repeatedly, and allows the production of a satisfactory amount of antibody within a relatively short period of time. Qualitatively, mouse peritoneal fluid is very similar to mouse serum and is as concentrated a source of precipitating antibody as is mouse serum(3).

Hiramoto(4) has reported that only 2-3 mg of total antigen is required per mouse for immunization, which is an obvious advantage compared to the amount required for the rabbit. The amounts of antigen given in our experiments, therefore, may have been higher than necessary for antibody production.

Summary. Antibodies to a sheep anterior pituitary (SAP) preparation and human chorionic gonadotrophin (HCG) have been developed in mouse ascites fluid. The ascites fluid was biologically assayed by the rat ovarian weight response to concurrently injected gonadotrophin. The anti-SAP ascites fluid contained antibody capable of completely blocking the biological activity of rat pituitary gonadotrophin. Likewise, the anti-HCG ascites fluid completely inhibited the activity of as much as 10 IU of HCG. Anti-HCG ascites fluid did not inhibit the biological activity of either SAP powder or rat pituitary gonadotrophin.

1. Munoz, J., Proc. Soc. Exp. Biol. and Med., 1957, v95, 757.
2. Munoz, J., Anacker, R. L., J. Immunol., 1959, v83, 640.
3. Anacker, R. L., Munoz, J., ibid., 1961, v87, 426.
4. Hiramoto, R., Bernecky, J., Jurand, J., Proc. Soc. Exp. Biol. and Med., 1962, v110, 895.
5. Herrmann, E. C., Jr., Engle, C., ibid., 1958, v98, 257.
6. Kasel, J. A., Lieberman, R., Smith, H. A., Virology, 1959, v9, 702.
7. Lieberman, R., Douglas, J. O. A., Humphrey, W., Jr., Science, 1959, v129, 775.
8. Lieberman, R., Douglas, J. O. A., Mantel, N., J. Immunol., 1960, v84, 514.
9. Lieberman, R., Mantel, N., Humphrey, W., Jr., Proc. Soc. Exp. Biol. and Med., 1961, v107, 163.
10. Lieberman, R., Mantel, N., Humphrey, W., Jr., Blakely, J., ibid., 1962, v110, 897.
11. Hashimoto, C., McShan, W. H., Meyer, R. K., Biochem. & Biophys. Res. Commun., 1965, v21, 120.
12. Duncan, D. B., Biometrics, 1955, v11, 1.
13. Thompson, K. W., Proc. Soc. Exp. Biol. and Med., 1937, v35, 640.
14. Marvin, H. N., Meyer, R. K., Endocrinology, 1943, v32, 271.
15. Deutsch, H. F., McShan, W. H., Ely, C. A., Meyer, R. K., Am. J. Physiol., 1950, v162, 393.
16. Snook, R. B., Cole, H. H., Endocrinology, 1964, v74, 52.
17. ———, ibid., 1965, v76, 20.

Received August 24, 1966. P.S.E.B.M., 1966, v123.

Induction of Hypothermia by Dimethyl Sulfoxide in Rats Exposed to Cold: Tissue and Enzyme Changes. (31623)

P. D. ALTLAND, B. HIGHMAN AND M. PARKER
Laboratory of Physical Biology and Laboratory of Experimental Pathology, National Institutes of Health, NIAMD, Bethesda, Md.

Recently we reported that injection of rats with dimethyl sulfoxide (DMSO) before exercise in a rotating cage markedly increased changes in serum enzyme levels attributable to exercise and increased fatty deposits in the heart, liver and striated muscle(1). Although DMSO apparently affected membrane permeability, the precise mechanism of such an effect was not known. We thought that further studies on the effects of DMSO on rats subjected to other stresses might clarify the nature of its effect on membranes. Exposure to cold was chosen for such a study.

After intraperitoneal injection of DMSO

body temperature falls in mice kept at room temperature(2) and in rats exposed to cold (3). We have studied physiological and histological effects produced by both topical and intraperitoneal injections of DMSO in rats exposed 5 hours to 1.7°C, a level of cold exposure without effect on body temperature of untreated rats.

Methods. Male Sprague-Dawley rats weighing approximately 250 g were placed individually in metal cages with coarse wire mesh flooring and exposed in a cold room at 1.7 ± 0.6°C for 5 hours without food or water. In the first experiment, about 5 minutes before exposure to cold, one-half of the rats received i.p. 4.5 g/kg DMSO* (10 ml/kg of a 41% aqueous solution). Rats from corresponding lots were used as controls. The numbers of rats used in each group are shown in Table I.

In a second experiment, the backs of the rats were shaved and moistened with 2 ml (about 6.5 g/kg) of a 90% aqueous solution of DMSO. In a control group the backs were moistened with water. After about 30 minutes, when the skin had dried, one-half of each group was placed for 5 hours in the cold room maintained at 1.7°C.

Microhematocrit determinations were made from tail blood and other tests on blood obtained by cardiac puncture under light ether anesthesia. Only one serum sample was obtained per rat. Serum values of the following enzymes were determined by spectrophotometric methods, previously described: glutamic oxalacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), aldolase (SAld), alkaline phosphatase (SAkP) lactic dehydrogenase (SLDH), and malic dehydrogenase (SMDH)(4,5). The isoenzymes of SLDH and SMDH were separated on cellulose acetate strips by electrophoresis and visualized by treatment with nitro blue tetrazolium as previously described(1,5). Serum urea nitrogen (SUN) was measured using deproteinized serum and the method described by Karr(6). Serum glucose was measured with glucose oxidase, using Glu-

* Obtained from Crown Zellerbach Corp., Camas, Wash.

TABLE I. Effects of Intraperitoneal Injection of Dimethyl Sulfoxide on Serum Enzymes, Urea Nitrogen, and Glucose of Rats Exposed to Cold.‖

Conditions	No. rats	SGOT	SGPT	SAld	SAkP	SLDH	SMDH	SUN	Glucose
Untreated									
Control	11	234 ± 11	67 ± 6	43 ± 5	82 ± 9	626 ± 72	599 ± 104	24.0 ± .8	125 ± 3
1.7°C, 5 hr	10	246 ± 23	73 ± 5	61 ± 13	35 ± 2*	731 ± 131	651 ± 155	36.5 ± 4.3*	129 ± 6
1.7°C, 5 hr + 19 hr	9	274 ± 15†	137 ± 20*	51 ± 7	39 ± 4*	465 ± 101	463 ± 91	20.9 ± 1.3	133 ± 5
1.7°C, 5 hr + 43 hr	5	227 ± 12	60 ± 6	20 ± 7	48 ± 10	472 ± 52	610 ± 112	20.2 ± 1.0	135 ± 4
DMSO treated									
DMSO, 5 hr	10	330 ± 49	72 ± 6	78 ± 17	80 ± 6	731 ± 105	483 ± 63	20.8 ± 1.2	141 ± 6
DMSO, 24 hr	10	268 ± 30	74 ± 3	56 ± 4	96 ± 7	580 ± 43	506 ± 48	24.0 .6	141 ± 6
DMSO 1.7°C, 5 hr	12	758 ± 65‡	246 ± 29‡	178 ± 21‡	40 ± 7*	3785 ± 445‡	2816 ± 289‡	45.1 ± 3.6*	347 ± 40‡
DMSO 1.7°C, 5 hr + 19 hr	12	539 ± 69‡	161 ± 20‡	101 ± 8§	51 ± 6*	544 ± 117	418 ± 43	21.6 .8	126 ± 7
DMSO 1.7°C, 5 hr + 43 hr	6	228 ± 20	64 ± 7	36 ± 4	26 ± 3*	433 ± 63	493 ± 72	23.7 ± 1.8	140 ± 9

* Significantly different from controls, $P < .01$ (t test).
† Significantly different from controls, $P < .05$ (t test).
‡ Significantly different from rats exposed to cold 5 hr, $P < .01$ (t test).
§ Significantly different from rats exposed to cold 5 hr, $P < .05$ (t test).
‖ Values expressed as mean ± S.E. SGOT, SGPT, SAld, SLDH, and SMDH are given in units/ml; SAkP in Bodansky units; SUN and glucose in mg/100 ml.

costat® reagent.† All results are expressed as mean values ± SE.

The rectal temperature of the rats was taken with a small animal thermistor probe (YSI #402) and a Tele Thermometer.‡

The heart, liver, kidney, adrenals and thigh muscles were fixed in a 10% aqueous solution of buffered formalin (pH 7.0). Frozen sections were stained for fat with oil red O. Portions of the liver, heart, and muscle of some rats were fixed in acetic-alcohol-formalin and stained for glycogen by the periodic acid-Schiff method(7).

Results. Effects of intraperitoneal injection of DMSO. In 7 rats maintained at room temperature DMSO lowered the mean body temperature from 38.2 ± 1.1 (SE) to 36.1 ± .2°C within 1 hour. No further significant change occurred in the next 4 hours. In 10 rats given DMSO and then exposed to 1.7°C, however, the body temperature declined from a mean value of 38.4 ± .2 to 32.6 ± .5, 30.7 ± .9, 28.3 ± 2, 27.9 ± 2 and 25.2 ± 2°C at 1, 2, 3, 4, and 5 hours, respectively. The body temperature of 10 untreated rats did not change significantly during a 5-hour exposure to 1.7°C.

None of 20 control rats given DMSO died or appeared ill. None of 31 rats given DMSO and exposed to cold died during the 5-hour exposure, but one died 19 hours later.

Immediately after a 5-hour exposure of untreated rats to 1.7°C there was a significant rise in SUN, but no change in serum glucose or enzyme values, except for a fall in SAkP (Table I). Nineteen hours later the SUN values were normal, but SGOT and SGPT values were elevated. All serum enzyme values were normal 43 hours after ending the cold exposure.

No changes in serum enzymes, SUN, or serum glucose were observed at 5 or at 24 hours following a single i.p. injection of DMSO in rats kept at 23°C (Table I). However, exposure of DMSO-treated rats to cold induced a marked hyperglycemia and a significant increase in all serum enzymes studied except SAkP, which remained at the low level resulting from cold exposure alone (Table I). Nineteen hours after ending the cold exposure, SGOT and SAld values in the rats treated with DMSO remained significantly above those in untreated rats. After another 24 hours all of the serum values of DMSO-treated rats exposed to cold were normal, except for a persistently low SAkP value.

Two rats of the DMSO-cold group autopsied at 5 hours showed excessive pleural fluid. The LDH concentrations in the pleural fluid (6300 and 9740 U/ml) were higher than the corresponding SLDH values (5440 and 5520 U/ml). All 5 LDH isoenzyme brands were intensified in the pleural fluid and in the serum.

Electrophoresis of the serum of 11 control rats for SLDH isoenzymes showed a well-defined band 5 and a less intensely stained band 1, similar to that previously reported (5). Two showed a faint band 2, and 4 showed faint bands 3 and 4. A 5-hour exposure to cold usually did not increase the number or intensity of the isoenzyme bands. One exceptional rat with an SLDH value of 1870 units showed 5 intensely stained isoenzyme bands. There were no significant changes in SLDH isoenzymes at 19 and 43 hours after ending a cold exposure. DMSO alone did not alter the number or intensity of SLDH isoenzymes at 5, 24, and 48 hours after injection.

All 5 SLDH isoenzyme bands were deeply stained within 5 hours in the DMSO-treated rats exposed to cold. After 19 hours rest at 23°C only bands 1, 2 and 5 remained well-defined, band 3 was present in only 2 of the 12 rats and none showed band 4. After 43 hours rest all 6 rats showed bands 1 and 5, and 2 rats still retained band 2.

The SMDH isoenzyme band associated with the cytoplasmic fraction was present in all rats. The isoenzyme band identified with the mitochondria was absent in all untreated rats even in those exposed to cold. All rats given DMSO and exposed 5 hours to cold, however, showed a prominent SMDH mitochondrial band. Only 1 of 12 rats retained this mitochondrial band after 19 hours at 23°C, and the band was absent in the 6 rats studied after another 24 hours.

† Worthington Biochemical Corp., Freehold, N. J.

‡ Yellow Springs Instrument Co., Yellow Springs, Ohio.

TABLE II. Incidence of Moderate to Severe Fatty Changes and Glycogen Depletion in Organs of Rats Given an Intraperitoneal Injection of DMSO and Exposed 5 Hours to Cold.

Condition	No./No. examined showing moderate to marked Fatty changes			Glycogen depletion		
	Liver	Heart	Muscle	Liver	Heart	Muscle
			Untreated			
1.7°C, 5 hr	0/10	0/10	1/10	10/10	4/9	3/9
1.7°C, 5 hr + 19 hr	0/9	0/9	0/9	0/9	7/9	1/9
1.7°C, 5 hr + 43 hr	3/6	0/6	0/6	0/6	5/6	4/6
			DMSO treated			
DMSO 1.7°C, 5 hr	1/12	1/10	0/10	12/12	8/10	9/10*
DMSO 1.7°C, 5 hr + 19 hr	8/12†	1/12	3/12	4/12	8/12	6/12
DMSO 1.7°C, 5 hr + 43 hr	3/6	0/6	0/6	0/6	3/6	3/6

* Significantly different from corresponding untreated group, $P < .05$ (chi-square test).
† Significantly different from corresponding untreated group, $P < .01$ (chi-square test).

During a 5-hour exposure to cold the rats lost an average of 19 g (7% total body weight). After resting 19 to 43 hours at 23°C, the body weight loss was 17 and 11 g, respectively (6 and 4% total body weight). The DMSO-treated rats exposed to cold lost an average of 28 g (9.5% of body weight) within 5 hours, and after resting 19 and 43 hours at 23°C the average weight loss increased to 34 and 40 g, respectively (11.6 and 13.6% of body weight).

Cold exposure did not change the mean hematocrit values of untreated rats, but the mean hematocrit value of 10 rats given DMSO before cold exposure increased from 46.2 ± 1.1 to 52.5 ± 1.3 within 5 hours (significant difference $P < .01$). After 19 hours at 23°C the mean hematocrit values had returned to control levels.

Pathologic findings. As reported previously (8), exposure of rats to cold for 16 hours often induced fatty changes in the liver, kidney, and heart and depletion of lipid in the adrenal cortex and of glycogen in the liver and muscle; no significant tissue changes were seen in routine paraffin sections. In this study (Table II), the incidence of fatty changes was significantly greater in the livers of DMSO-treated rats than in untreated controls 19 hours after cold exposure. No significant fatty changes were noted in the kidney, and only occasional animals showed slight fatty changes in the heart or muscle or slight depletion of lipid in the adrenal cortex. There was complete depletion of glycogen in the liver of all animals at the end of the 5-hour exposure. There also appeared to be a moderate to marked depletion of glycogen in the heart and muscle of some of these animals immediately and 19 hours after exposure. Repletion of glycogen was complete in the liver 43 hours after exposure, but only partial in the heart and muscle of some of the animals (Table II). No other significant changes were found.

Exposure to cold for 5 hours caused hemoglobinemia in 1 of 24 untreated rats and in 3 of 31 DMSO-treated rats. The serum values of rats with hemoglobinemia were similar to those without such evidence of hemolysis, except for elevated SLDH values.

Effects of topical application of DMSO. The mean body temperature of 8 shaven DMSO-treated rats increased from 38.4 ± 0.1 to 38.8 ± 0.1°C during the 30-minute drying period before exposure to cold. During the first hour of exposure to cold the mean body temperature dropped to 37.2 ± 0.3°C, and reached a low of 36.7 ± 0.2°C at 5 hours. The mean body temperature of 8 shaven DMSO-treated rats not exposed to cold increased from 38.1 ± 0.04 to 38.8 ± 0.1°C during the drying period, then returned to 38.1 ± 0.2°C at 1 hour and gradually declined to 37.1 ± 0.3°C at the end of 5 hours at 23°C. Eight shaven rats wet with water showed no change in body temperature during the drying period, but during the 5-hour cold exposure their mean body temperature also declined gradually from 38.6 ± 0.2 to 36.5 ± 0.5°C. A group of 8 shaven rats treated with water and not exposed to cold had normal

body temperature throughout a 5-hour period of study.

A 5-hour cold exposure of 6 untreated shaven rats produced a significant increase ($P<.01$) in SGOT (271 ± 19 to 351 ± 15 units/ml), SAld (54 ± 8 to 90 ± 10 units/ml), and SUN (26.2 ± 2.9 to 32.1 ± 1.7 mg/100 ml). There were no other significant changes in serum enzymes, isoenzymes or glucose. Topical treatment of rats with DMSO prior to exposure to cold did not alter significantly the changes in serum values attributable to cold exposure alone. No histological study was made of the skin of topically-treated rats, but a study of the heart, liver, kidney, and thigh muscles revealed no abnormalities.

Discussion. In this study DMSO markedly increased values of SGOT, SGPT, SAld, SLDH, SMDH, and SUN in rats exposed 5 hours to 1.7°C. Such an exposure without DMSO did not elevate any serum enzymes except transaminases at 19 hours, and, as previously reported(8), even more severe exposure (two 5-hour exposures of wet rats to 1.7°C) produced increases only in SGOT, SGPT, and SAld. Apparently the enhanced effect of DMSO on enzyme levels is not limited only to those enzymes affected by the particular stress in untreated rats, as lactic and malic dehydrogenase values were not affected by cold alone.

Our histologic and isoenzyme studies support the concept that DMSO augments the permeability of cellular and mitochondrial membranes of many organs and tissues of rats exposed to cold, and that the incidence of tissue changes may provide an index of the severity of the altered permeability. For example, in the DMSO-treated rats exposed to cold, the accentuation of SLDH isoenzyme band 1, the major isoenzyme in the rat heart, suggests increased liberation of LDH from the rat myocardium(5). Similarly, a prominent band 5 suggests increased liberation of LDH from the liver, muscle or erythrocytes, and prominent bands 2, 3, and 4 suggest increased contributions from other organs. The presence of the mitochondrial MDH isoenzyme band in the serum of all DMSO-treated rats exposed to cold and its absence in those not given DMSO suggests that DMSO increases the permeability of mitochondrial membranes. Marked histological changes in the heart, liver, and muscle tended to be earlier in onset and higher in incidence in the DMSO-treated rats exposed to cold. The high incidence of tissue changes, particularly in the liver, 19 hours after the end of the cold exposure (Table II), correlates well with the elevated serum enzyme levels at this period.

A marked hyperglycemia was found in DMSO-treated rats at 5 hours after beginning cold exposure (Table I). The histological studies revealed marked depletion of hepatic glycogen in both groups at 5 hours and a greater loss of glycogen in the muscle of the DMSO-treated rats. Repletion of glycogen in the liver was delayed in DMSO-treated rats (Table II). These findings indicate that DMSO may affect certain mechanisms in carbohydrate metabolism and suggests the need for caution in the use of DMSO in diabetic patients.

The mechanism of action of DMSO on the cell membrane may be either direct or indirect or both. A direct effect is suggested by recent evidence that DMSO influences lipoperoxidation in the cell membrane, a process considered to affect cellular permeability(9). It is also possible that DMSO may indirectly affect the levels of serum enzymes by reducing the body temperature. Previously we have shown that the changes in serum enzymes were apparently greater in rats with a more pronounced hypothermia(8). DMSO may also act indirectly by influencing adrenal hormones, which may in turn affect cellular membrane permeability. Large doses of epinephrine increase the concentration of serum enzymes in dogs and rats(10). Cold exposure results in an increased release of epinephrine (11), as well as cortical hormones(12). Corticosterone alone is ineffective in increasing release of serum enzymes, but is thought to potentiate epinephrine-induced serum enzyme elevations(13). Another possible factor involved in the effects of DMSO cold-exposure on membrane permeability may be anoxemia. Stagnant anoxemia is produced in dogs with hypothermia(14). Moreover, hypoxia also causes serum enzyme changes which vary

with the degree of hypoxia and are markedly augmented by DMSO(15).

SAkP significantly declined in rats after exposure to 1.7°C. DMSO given i.p. or topically did not alter this effect of cold. The mechanism by which cold and other stresses interfere with the normal release of SAkP is unknown.

We have found a 4% loss in body weight after 5 hours of cold exposure, and a significant increase in hematocrit in DMSO-cold exposed rats, suggesting hemoconcentration. Possibly DMSO is an important factor contributing to hemoconcentration, since no change in hematocrit was found in rats exposed to 1.7°C alone.

Intraperitoneal injection of 4.5 g/kg DMSO in mice lowered the body temperature about 4.2°C within 1 hour(2). This same i.p. dose of DMSO in rats lowered the body temperature only 2.1°C within 1 hour. Topical application of either DMSO or water on shaved rats before exposure to cold resulted in a decline in body temperature of about 2°C in 5 hours. The transient elevation in body temperature preceding the development of hypothermia after topical treatment with DMSO has not been reported previously.

The reason for the marked hypothermia in rats given an intraperitoneal injection of DMSO and exposed to cold is not known. Generally, exposure to cold increases heat production, due to shivering. Since it did not prevent this shivering, it is possible that DMSO may inhibit heat production by directly or indirectly affecting metabolism. The ability of other drugs such as chlorpromazine, reserpine, and norepinephrine to decrease the body temperature of rats has been considered to be due to their effects on the metabolic rate(17). Further study on the effect of DMSO on metabolism seems warranted.

Summary. Topical and intraperitoneal treatment of rats with DMSO produced a 1 to 2°C drop in body temperature in 5 hours at 23°C; however, those treated topically showed an early transient rise of as much as 0.7°C. No serum enzyme, urea nitrogen, or sugar changes were produced by DMSO at 23°C. The only serum enzyme changes noted during a 5-hour exposure to 1.7°C was a fall in SAkP levels, but 19 hours later a rise in serum transaminases became evident. DMSO given intraperitoneally prior to cold exposure caused a marked decrease in body temperature and a marked increase within 5 hours in all serum enzymes studied, including SGOT, SGPT, SAld, SLDH, and SMDH. DMSO did not influence SAkP values. Also, the DMSO-treated rats exposed to cold showed intensification of all 5 SLDH isoenzyme bands and the isoenzyme band of SMDH associated with mitochondria and an increased elevation in SUN, serum glucose and hematocrit. At 5 hours, all rats exposed to cold showed marked depletion of hepatic glycogen, but repletion was delayed in the DMSO-treated group. Nineteen hours after exposure to cold, only depletion of liver glycogen and fatty changes in the liver, heart, or muscle persisted. Topicaly DMSO before exposure to cold did not alter the serum enzyme values or tissue changes produced by cold exposure alone. These findings show that intraperitoneal injection of DMSO in rats exposed to cold induces marked hyperglycemia, a severe hypothermia accompanied by a marked rise in serum enzyme values and glycogen depletion, and an increased incidence of fatty changes in the liver and muscle.

The authors gratefully acknowledge the advice and technical assistance of Dr. B. Dean Nelson and Edwin C. Thompson.

1. Altland, P. D., Highman, B., Nelson, B. D., Garbus, J., Life Sci., 1966, v5, 375.

2. Ashwood-Smith, M. J., Intern. J. Radiation Biol., 1961, v3, 41.

3. Panuska, J. A., Malinin, T. I., Mentz, R. J., Fed. Proc., 1966, v25, 1446.

4. Highman, B., Altland, P. D., Am. J. Physiol., 1963, v205, 162.

5. Garbus, J., Highman, B., Altland, P. D., ibid., 1964, v207, 467.

6. Karr, W. G., J. Lab. Clin. Med., 1924, v9, 329.

7. Lillie, R. D., Histopathologic technic and practical histochemistry, Blakiston Co., N. Y., 1954.

8. Highman, B., Altland, P. D., Proc. Soc. Exp. Biol. and Med., 1962, v109, 523.

9. Muset, P. P., Martin-Esteve, J., Experientia, 1965, v21, 649.

10. Highman, B., Altland, P. D., Garbus, J., Arch. Path., 1965, v80, 332.

11. McComisky, D. M., Beaton, J. R., Can. J. Biochem. Physiol., 1963, v41, 2038.

12. Boulouard, R., Fed. Proc., 1963, v22, 750.

13. Pearl, W., Balaza, T., Buyske, D. A., Life Sci., 1966, v5, 67.
14. Fisher, B., Russ, C., Fedor, E. J., Am. J. Physiol., 1957, v188, 473.
15. Highman, B., Altland, P. D., Life Sci., 1966, v5, 1839.
16. Sellers, E. A., You, R. W., Thomas, N., Am. J. Physiol., 1954, v177, 372.
17. Johnson, G. E., Sellers, E. A., Schönbaum, E., Fed. Proc., 1963, v22, 745.

Received June 23, 1966. P.S.E.B.M., 1966, v123.

Induction of Interferon in Mice Infected with *Toxoplasma gondii.** (31624)

MICHAEL W. RYTEL† AND THOMAS C. JONES (Introduced by E. D. Kilbourne)
Division of Virus Research Department of Public Health and Tropical Medicine Unit, Division of Allergy and Infectious Disease, Department of Medicine, Cornell University Medical College, New York

Since the observation of Rotem *et al*(1) that interferon can be elicited by non-viral nucleic acids, the recognized number of such non-viral inducers of interferon has steadily increased. About 15 of these inducers have been described. Some of them are macromolecular compounds(2,3), others are microorganisms(4).

Because *Toxoplasma gondii* is an obligate, intracellular parasite, which, *in vivo*, multiplies in cells of the reticuloendothelial system (known to be a rich source of interferon(5)) it was of interest to determine whether *T. gondii* is capable of inducing interferon.

Materials and methods. The parasite. The RH strain(6) of *T. gondii* was maintained in CFW, 3-4-week-old male mice by serial intraperitoneal passage every 4 and 3 days alternately. At the time of each passage the peritoneal exudate was cultured for bacteria in thioglycollate broth, diluted 1:20 with isotonic saline, and inoculated intraperitoneally into healthy mice (0.2 ml per mouse).

Infection of cell cultures. Saline suspensions of peritoneal exudate containing *T. gondii* were centrifuged at 55 *g* for 5 minutes. The supernate was removed, centrifuged at 700 *g* for 10 minutes, and the sediment resuspended in Eagle's minimum essential medium (MEM). The *T. gondii* concentration (as determined by direct counting in a hemocytometer) was 16×10^6 per ml. The suspension was then diluted further 100 × in MEM with 20% heat inactivated calf serum. Clone L-929 (murine fibroblast) cells (ATCC) were grown in 60 mm plastic petri dishes (Falcon) in M199 with 5% fetal calf serum. The cultures were infected with the parasite approximately 1 day prior to reaching a complete monolayer (*i.e.*, 50-100 cells per microscopic field, at 450× magnification).

Infection of mice. Peritoneal exudate from mice infected 3 days previously with toxoplasma, was diluted 1:20 with saline and inoculated intraperitoneally into 3-4-week-old CFW male mice. The exudate was cultured in thioglycollate broth. No bacteria were recovered. A portion of the inoculum was filtered through an 0.45 Millipore filter and applied to murine fibroblast (L-929) monolayers. No cytopathogenic effect was observed. In addition, when cells pretreated in this manner were challenged with vesicular stomatitis virus (VSV) (Indiana strain), there was no inhibition of viral multiplication. These observations weigh against the presence of viral agents capable of multiplication or direct interference in the murine cell system employed. Each mouse received either 0.2 ml of toxoplasma suspension or 0.2 ml of the control solution. At different

* Supported in part by Research Grant AI-01595 and Training Grant 5-T1AI6 (Dr. Rytel) and Training Grant AI-255 (Dr. Jones) from Nat. Inst. of Allergy & Infect. Dis., NIH, USPHS, Bethesda, Md.

† Present address: Dept. of Medicine, Univ. of Tennessee, Memphis.