produced profound changes in the metabolic pattern of the body, these antibiotics should not be used indiscriminately.

Summary. Oxytetracycline or tetracycline, 10 mg/kg, was administered for 10 consecutive days to rabbits and rhesus monkeys. Glucose tolerance test was performed and different fractions of serum lipids were estimated in these animals before and after treatment with the antibiotics to find if their prolonged use interfered with carbohydrate and lipid metabolism. Both antibiotics diminished glucose tolerance. The pattern of serum lipids was changed. There were increases in the serum triglycerides, phospholipids, β-lipoprotein cholesterol and free fatty acids in most of the animals after treatment with the antibiotics. Tetracyclines should be used with caution due to the metabolic disturbances they might produce.

1. Banerjee, S., Sampath Kumar, K., Bandyopadhyay, A., Proc. Soc. Exp. Biol. & Med., 1966, v122, 652.

2. Hagedorn, H. C., Jensen, B. N., Biochem. 2., 1923, v137, 92.

3. Bandyopadhyay, A., Banerjee, S., Proc. Soc. Exp. Biol. & Med., 1963, v114, 222.

4. Banerjee, S., Sampath Kumar, K., J. Sci. Indust. Res., 1962, v21C, 169.

5. Klimov, A. N., Kirov, S. M., Antibiotiki Eksptl-Klin. Tzuchen., Sobornik Robot. p103, 1959.

6. Van Meter, J. C., Oleson, J. J., Science, 1951, v113, 273.

7. Hunter, F. E., Jr., Lowry, O. H., Pharmacol. Rev., 1956, v8, 89.

8. Arora, K. L., Krishna Murti, C. R., J. Sci. Indust. Res., 1954, v13B, 482.

9. Zimmerman, H. J., Humoller, F. L., Am. J. Physiol., 1953, v175, 468.

Received March 9, 1967. P.S.E.B.M., 1967, v125.

A Rapid Screening Method for Detection of T Antigens in Sera of Tumor-Bearing Hamsters.* (32162)

RICHARD M. JAMISON†
(Introduced by N. G. Anderson)
Molecular Anatomy Section, Biology Division, Oak Ridge National Laboratory,‡ Oak Ridge, Tenn.

Cells present in hamster tumors induced by papovavirus SV-40 contain new cellular antigens (tumor or T antigens). These T antigens were first discovered by *in vivo* immunity tests(1). Similar antigens have been found in hamster tumors induced by human adenoviruses(2). Hamsters bearing tumors induced by either SV-40 or by oncogenic human adenoviruses develop circulating antibody capable of reacting with their respective T antigens *in vitro*. The presence of T antigens in cells transformed *in vitro* and in cells infected with the virus during the early stages of viral replication has been demonstrated by the techniques of complement fixation (CF) and immunofluorescence by using sera from tumor-bearing hamsters(2-9). Good correlation between immunofluorescence and CF antigen titers has been reported(10).

Use of particles of lecithin and cholesterol as inert carriers of viral antigens in agglutination tests with specific antisera has been recently documented. We have modified the basic methods of Klein et al(11) to demonstrate antibody response to the T antigens of papovavirus SV-40 and human adenovirus type 31 by an agglutination technique. The reactions are type specific. Results obtained in screening sera from tumor-bearing hamsters for the presence of T antibodies by utilizing the agglutination technique compare favorably with those obtained by the CF technique.

Materials and methods. A. Tumor antigens. The SV-40 T antigens utilized were from

* Research sponsored by the Joint NIH-AEC Molecular Anatomy Program which is supported by Nat. Cancer Inst., Nat. Inst. of Allergy & Infect. Dis., and Nat. Inst. of General Med. Sciences.

† Present address: Dept. of Pathology, School of Med., Univ. of Colorado Medical Center, Denver.

‡ Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.

several different sources:

(1) A 20% extract of transplanted SV-40 tumors (virus-free) available commercially§. This material had a CF titer of 1:4 *vs* 4 units of homologous antiserum.

(2) Hamster tumor cells‖ were cultured *in vitro,* washed with phosphate-buffered saline (PBS) (pH 7.0), scraped from the flask, centrifuged and resuspended in PBS. This suspension was then alternately frozen rapidly and thawed in a cycle repeated three times. The cellular debris was then removed from the antigen preparation by low-speed centrifugation. Various lots of antigen prepared in this manner had titers of 1:4-1:8 in CF tests *vs* homologous antisera.

(3) A 10% extract of *in vitro* transformed cells line WI 180.¶ This preparation had a CF titer of 1:64 *vs* homologous antisera.

Human adenovirus type 31 T antigen was prepared as a 10% extract of hamster tumors prepared in Miller-Golder buffer(12). The original hamster tumor was obtained commercially§ and was passaged 8 times in weanling hamsters in this laboratory before being used in these experiments.

The control tumor antigen used throughout this study was a commercially available§ 20% extract of the spontaneous non-viral hamster tumor 173-FSAB. The CF titer of this preparation was <1.2 *vs* antisera from hamsters bearing tumors induced by either SV-40 or adenovirus type 31.

B. Tumor antisera. Antisera used in this study were obtained from a variety of sources. Reference antisera to T antigens of both human adenovirus 31 and papovavirus SV-40 were obtained commercially.§ In addition, sera were collected at this laboratory from hamsters bearing transplants of tumors induced by either SV-40 or Adenovirus type 31. Serum collected from individual hamsters was screened separately by both the CF and agglutination techniques before being pooled. Control normal hamster serum was obtained as a pool.** Other control sera were obtained from the hamster colony of our laboratory. All sera were heated at 56°C for 30 minutes prior to testing by either agglutination or CF.

C. Complement fixation techniques. The microtechnique of Sever(13) as modified by Huebner *et al*(2) was utilized for all CF tests.

D. Preparation of reagents for agglutination tests. The methods of Klein *et al*(11) were slightly modified for preparation of all reagents. A solution containing 0.1% lecithin†† and 0.9% cholesterol‡‡ in absolute ethanol was prepared; then 0.3 ml of this solution was added drop-by-drop into a glass vial containing 0.6 ml of the appropriate antigen. The vial was rotated during the addition of the lecithin-cholesterol solution and then shaken vigorously for 1 minute; then 1.1 ml of 0.9% PBS was added, and the vial again shaken vigorously for 1 minute. The mixture was then centrifuged for 15 minutes at approximately 3,000 rpm, and the supernatant fluid was discarded. The sediment was suspended in 1.9 ml of PBS.

Unless it is to be used immediately, the antigen preparation should be sealed in small glass vials. Preparations opened and transferred to test tubes for use should be kept tightly stoppered. We have observed reduction in activity of T antigen preparations kept in stoppered test tubes for periods of 1 week. Klein *et al* state that preparations of influenza antigens prepared in a similar manner and sealed in glass vials are stable for periods up to 1 year.

Performance of the agglutination tests. Agglutination tests were carried out in a standard Klein agglutination plate. Two drops of the serum to be tested and the 1 drop of antigen were added to each well, and the fluid was mixed and incubated at 4°C for 20 minutes. The plates were gently rocked every 5 minutes. The test was read by use of microscope at a magnification of approximately 50 ×.

Known positive and negative controls must be included in all tests.

Results and discussion. In positive tests, agglutination of the lecithin-cholesterol par-

§ Flow Laboratories, Rockville, Md.

‖ Hamsters bearing SV-40 tumors were obtained originally from Dr. B. E. Eddy and Dr. D. Axelrod Nat. Cancer Inst., Bethesda, Md.

¶ This antigen was furnished by Dr. D. Axelrod Nat. Cancer Inst., Bethesda, Md.

** Hyland Laboratories, Los Angeles, Calif.

†† Sylvana Chemical Co., Orange, N. J.

‡‡ Difco Laboratories, Detroit, Mich.

TABLE I. Comparison of Results Obtained by CF and Agglutination Tests of Sera from Tumor-Bearing Hamsters.

T antigen:	Adenovirus Type 31		Papovavirus SV-40	
Tests:	CF	Agglutination	CF	Agglutination
Positive	25	24	15	13
Negative	3	5	5	7
Questionable*	1	0	2	2†

* CF titer = 1:2.

† These two sera are not the same 2 sera represented as questionable by CF. Both "CF-questionable" sera were negative by the agglutination test.

ticles by antisera is readily visible in the microscope. This agglutination can arbitrarily be graded 1-4+. The degree of reaction is variable depending on the antigen preparation and the antiserum used in the test (*i.e.*, some antigen preparations form large macroscopic aggregates when tested against some positive sera. Such sera may elicit the formation of only microscopic aggregates from other antigen preparations.) The relative reactivity noted (*i.e.*, 1-4+) does not seem to be directly related to the amount of antibody (expressed as C′ fixing units) present in the serum or to the specific antigen preparation used.

Ocasionally sera will non-specifically agglutinate the lecithin-cholesterol particles. For this reason, sera shoud always be tested with a known non-specific antigen preparation (negative control test).

Klein *et al*(11) report that antisera that are anticomplementary in CF tests still give specific reactions in the agglutination test against influenza antigens. It has been our experience that anticomplementary sera yield only questionable results when tested against T antigen preparations.

The correlation between the results obtained with the agglutination test and the CF test is shown in Table I. The results were obtained as blinds (*i.e.*, the technician did not know which sera were positive). Results such as these, coupled with tests of known sera, indicate that the agglutination test is quite specific. We have not observed false positive reactions. However, we have performed tests which gave no clear results. Such tests were recorded as "questionable." The sera which yield questionable results when tested against a particular preparation of T antigen can be retested with other preparations of the same antigen and frequently give a definite result (*i.e.*, either positive or negative). In addition, the incubation of questionable reaction mixtures at 37°C will occasionally allow the performance of a definite test. It must be pointed out that non-specific reactions may occur when the antigen-antibody mixtures are incubated at 37°C.

The agglutination test, although specific, is slightly less sensitive than the CF test. Table II demonstrates typical results of comparative titrations. It can be seen that titrations of antisera by the agglutination technique frequently yield titers less than those obtained by the CF test.

The conjugation of T antigens to lecithin-cholesterol particles has not been shown to be quantitative. Table III demonstrates typical data. It can be seen that only enough antigen to cause an 8-fold drop in CF tests was removed from an antigenic solution by the

TABLE II. Comparison of Results Obtained by Titration of Antisera by CF and Agglutination.

Sera	Titer CF	Titer Agglutination
Anti-SV-40 T	1:8	1:8
	1:4	1:4
	1:8	1:8
	1:16	1:4
	1:64	1:4
	Anticomp.*	?
	<1:2	0
Anti-Adenovirus Type 31 T	1:4	0
	>1:64	>1:32
	1:64	1:8
	1:8	1:16
	1:64	1:8
	1:32	1:16

* Anticomplementary.

TABLE III. Results of Titration of Tumor Antigen Preparations Before and After Conjugation to Lecithin-Cholesterol.

Adenovirus Type 31 antigen	CF titer against 4 units of anti-adenovirus 31 sera
Before conjugation	1:512
Supernatant after conjugation	1:64
Lecithin-cholesterol antigen	Anticomplementary

lecithin-cholesterol. Several alternative explanations for this fact can be offered:

(1) The tumor antigen preparations contain only one antigen which may exist in different physical states, only some of which can be conjugated to lecithin-cholesterol by the method used.

(2) The tumor antigen preparation contains multiple antigens some of which cannot be conjugated to lecithin-cholesterol particles.

(3) Excess tumor antigen was present during the conjugation procedure, and all available attachment sites on the lecithin-cholesterol particle were utilized. As obviously unagglutinated particles of lecithin-cholesterol may remain in the test solution after the conclusion of immune aggregation tests, we believe this to be improbable.

Summary. A rapid screening test for detection of antibodies to tumor antigens of papovavirus SV-40 and human adenovirus type 31 is described. The procedure is equally specific though slightly less sensitive than the CF test.

1. Habel, K., Eddy, B., Proc. Soc. Exp. Biol. & Med., 1963, v113, 1.
2. Huebner, R. J., Rowe, W. P., Turner, H., Lane, W., Proc. Nat. Acad. Sci., 1963, v50, 379.
3. Black, P. H., Rowe, W. P., Turner, H. C., Huebner, R. J., ibid., 1963, v50, 1148.
4. Rapp, F., Butel, J. S., Melnick, J. L., Proc. Soc. Exp. Biol. & Med., 1964, v116, 1131.
5. Pope, J. H., Rowe, W. P., J. Exp. Med., 1964, v120, 121.
6. Sabin, A. B., Shein, H. M., Koch, M. A., Enders, J. F., Proc. Nat. Acad. Sci., 1964, v52, 1316.
7. Shimojo, H., Yamamoto, H., Yoshikawa, E., Yamashita, T., Jap. J. Med. Sci. Biol., 1966, v19, 9.
8. Rapp, F., Kitahara, T., Butel, J. S., Melnick, J. L., Proc. Nat. Acad. Sci., 1964, v52, 1138.
9. Riggs, J. L., Takemori, N., Lennette, E. H., Proc. Soc. Exp. Biol. & Med., 1964, v120, 832.
10. Kitahara, T., Butel, J. S., Rapp, F., Melnick, J. L., Nature, 1965, v205, 717.
11. Klein, M., Chaefsky, S., Muller, M., J. Immunol., 1966, v97, 131.
12. Miller, G. L., Golder, R. H., Arch. Biochem., 1950, v29, 420.
13. Sever, J. L., J. Immunol., 1962, v88, 320.

Received March 10, 1967. P.S.E.B.M., 1967, v125.

Effect of Hypothalamic Deafferentation on Lactation in Rats.* (32163)

A. YOKOYAMA,† BELA HALÁSZ‡ AND CHARLES H. SAWYER
Department of Anatomy and Brain Research Institute, UCLA School of Medicine, Los Angeles, Calif.

Although it has been widely accepted that the suckling stimulus induces release of both prolactin from the anterior pituitary and oxytocin from the neurohypophysis, the pathway or pathways by which the suckling stimulus attains the hypothalamic areas controlling the release of these hormones has not yet been clarified. Recently considerable evidence has been reported(3,12) suggesting selective impairment of oxytocin secretion in hypothalamic lesions in lactating cats and rats. On the other hand, Averill and Purves (2) and Averill(1) reported selective blockade of prolactin secretion by lesions lateral to the paraventricular nuclei.

"Deafferentation" of the hypothalamus with a special knife designed by Halász and Pupp (7) presented itself as a good way to investigate the pathways by which the suckling stimulus causes secretion of the pituitary hormones influencing lactation. The present paper deals with preliminary observations on the effects of hypothalamic deafferentation on lactation in rats.

Methods and materials. Lactating Sprague-

* Supported by grants from Nat. Inst. Health (NB 01162) and Ford Foundation.

† Ford Foundation Fellow. Present address: Faculty of Agriculture, Nagoya Univ., Chikusa-ku, Nagoya, Japan.

‡ Ford Foundation Fellow. Present address: Inst. of Anatomy, Pecs Univ., Pecs, Hungary.