gesting that the activation by CCl_4 could be attributed to the split of the linkages between the enzyme and the lipids as observed when milk(10) and liver xanthine oxidase(2) are treated by other organic solvents.

Summary. Quantitative determinations of xanthine oxidase and dehydrogenase activity in blood serum of normal rats were made. It was shown that a single injection of 0.1 ml CCl_4/100 g body weight is able to activate the blood xanthine oxidase and dehydrogenase, reaching the highest values 20-25 hours after injection. However, after 35 hours the values returned to normal. Activation of the enzyme was also obtained *in vitro* by adding CCl_4 to a sample of serum of known enzyme content.

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Received October 13, 1955. P.S.E.B.M., 1955, v90.

Recently Classified Types of Coxsackie Virus, Group A. Behavior in Tissue Culture. (22088)

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In addition to the 10 previously described types of Coxsackie virus, Group A(1,2), 9 new types have now been assigned numbers. The representative strain of Type 11 is the Belgium 1 strain of Godenne and Curnen (3), that of Type 12, the Texas 12 of Contreras, Barnett, and Melnick(4). Type 13 was isolated in this laboratory from fecal specimens from Mexico. Type 19 was received from Dr. R. J. Heubner, and the remainder from Dr. J. H. S. Gear of the South African Institute for Medical Research(5). All are viruses which induce flaccid paralysis in suckling mice but no apparent disease in older mice and are characterized by the production of marked muscle degeneration but no significant lesions in the central nervous system in the experimental animal. With the exception of Types 12 and 14 all resemble Types 1, 7, and 9 in low infectivity of brain suspensions for suckling mice. Passage and testing with muscle suspension are necessary. In neutralization tests in suckling mice there is definite, although moderate, reciprocal cross reactivity of A-11 with A-15 and of A-13 with A-18. As noted by Contreras(4), A-12 cross reacts with A-5. Regular cross reactions may also be found with A-8 and A-12, more observable when brain tissue is used in neutralization tests. Slight cross reactivity of A-14 in A-7 serum, A-15 in A-17 serum, and A-17 in A-11 serum has been seen.

Tissue culture. Methods. Human uterine tissue and trypsinized monkey kidney tissue cultures were prepared as previously described (6). Trypsinized monkey testicular cultures were made similarly to the kidney cells. HeLa cell cultures were supplied by the Laboratories for Virology under the direction of Dr. Irving Gordon. On receipt, the nutrient fluid containing human serum was removed and replaced by 1.5 ml of the maintenance fluid of Scherer, Syverton, and Gey(7) without added serum. The tubes were rotated so that the entire inside surface was washed and were then allowed to stand upright for a minimum of one-half hour. The washings were removed and replaced by 1.0 ml of mainten-

TABLE I. Cytopathogenicity of Coxsackie Virus.

Coxsackie virus Group	Type	Monkey cells trypsinized Kidney	Monkey cells trypsinized Testes	Human cells Uterine plasma clot	Human cells HeLa cells
A	9	+	+	+	—
	11	—	—	+	+
	13	—	—	+	+
	15	—	—	+	+
	18	—	—	+	+
B	1	+	+	—	+
	2	+	+	—	*
	3	+	+	+	+
	4	+	+	—	(+)
	5	+	+	+	+

* Only one strain regularly cytopathogenic under the given conditions.
(+) More erratic than B-1, 3, and 5.

ance fluid. All types not cytopathogenic for these cells were retested on HeLa cells that had been given two further washings of 1.0 ml each. No additional cytopathogenic strains were encountered. In early tests 10% chicken serum was used in the maintenance fluid for the test and later, 2% calf serum. For many of the results recorded no serum was added. Three tubes each were inoculated with 0.1 ml of the test material. For the cultures grown on glass the inoculum was added to the tube after the maintenance fluid; for plasma clot cultures the tissue was inoculated and let stand at room temperature for one-half hour before the addition of the maintenance fluid. Neutralization tests were made, in general, as previously described(6).

Results. Trypsinized monkey kidney cell cultures. All types of Group B were cytopathogenic for monkey kidney cells, but only Type 9 of Group A(8,9). None of the newer Group-A types induced evident cell damage (Table I).

Trypsinized monkey testicular tissue cultures. In previous tests on stationary plasma clot cultures of monkey testicular tissue with 3 strains of A-7, 2 of A-9, one each of B-1, B-4, and B-5, and 6 of B-2, cytopathogenic activity was obtained with the A-9 strains only. Steigman obtained cell damage with 2 strains of B-2 on roller tubes of plasma clot cultures with fluid 199 as maintenance medium(10). Using the trypsinized cells, we have found that the same types cytopathogenic for monkey kidney cells induce cell damage in the testicular tissue grown on glass. With the Group-B types the effect was more prompt and more marked. The newer Group-A types were not cytopathogenic.

Human uterine tissue (plasma clot). Among the previously numbered types Group A, Type 9, some strains of Group B, Type 3, and Type 5(6) were found to be cytopathogenic for human uterine tissue. Four of the new types in Group A, Types 11, 13, 15, and 18, are also cytopathogenic. Cell damage may appear in from 3 to 7 days but is usually definite at 5 days. All are neutralized by their specific immune sera. Two of 4 strains of A-14 caused minor cell damage in early passages on this tissue and also on plasma clot cultures of monkey testicular tissue. This, however, could not be established on passage.

HeLa cells. Our experience with the previously numbered types has been, in general, similar to that of Crowell and Syverton(11). Group A, Types 1-10, were not cytopathogenic. Strains of Group B, Types 1 and 3, are cytopathogenic, as is Type 5. More difficulty was experienced with B-4. In earlier trials reactivity was erratic but more recently we have been able to make type determinations of a number of strains on HeLa cells, although

TABLE II. Neutralization Test in HeLa Cell Tissue Culture Coxsackie Virus, Group A, Type 15.

Test dose: Virus	Serum: Kind	Serum: Dilution	A-15 No. 52108: 5 days	A-15 No. 52108: 6 days
10^{-2}	Maintenance medium		2+ 2+ 2+	4+ 4+ 4+
10^{-3}			2+ 2+ 2+	4+ 4+ 4+
10^{-4}			— ? —	2+ 4+ 2+
10^{-5}			— — —	— — —
10^{-1}	Normal hamster	5	2+ 2+ 2+	3+ 4+ 4+
	A-11	5	— ? —	1+ 3+ —
		10	— — 1+	2+ 2+ 3+
		20	2+ 2+ 2+	4+ 4+ 4+
	A-15	10	— — —	— — —
		40	— — —	— — —
		160	— — —	— — —
		640	2+ 1+ ?	4+ 3+ 3+
Uninoculated controls			— — —	— — —

— = same as control uninoculated tissue; ? = slight damage, specificity doubtful; 1+ = small but definite area of damage; 2+ = large areas of definite damage but with large remaining areas of normal cells; 3+ = extensive destruction; 4+ = complete destruction.

endpoint titers are not high. With B-2 only one strain of 10 tested was consistently cytopathogenic on HeLa cells. Two other strains continue to give erratic results on repeated transfer. Among the new Group-A types the 4 which are cytopathogenic for human uterine cells, Types 11, 13, 15, and 18, are also cytopathogenic for HeLa cells. Beginning cell damage may be seen in from 2 to 6 days. Satisfactory neutralization tests may be made with these strains. The cross reactivity noted in the mouse neutralization tests is also evident in tissue culture (Table II).

Summary. Nine additional types of Coxsackie virus, Group A, have been assigned numbers, Types 11-19. Strains of 4 of these types, Types 11, 13, 15, and 18 are cytopathogenic for human uterine tissue in plasma clot culture and for HeLa cells, but not for trypsinized monkey kidney or testicular cells.

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Received October 17, 1955. P.S.E.B.M., 1955, v90.

Association of I^{131}-Labeled Lactogenic Hormone with Cytoplasmic Nucleoprotein of Mammary Gland Cell.* (22089)

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It has been reported from this laboratory (1) that lactogenic hormone labeled with I^{131} becomes associated predominately with the cellular particulates following intraductal injection into the rabbit mammary gland. It was suggested that this study indicates the importance of the mitochondria and microsomes in the synthetic activity associated with milk secretion and that the site of action of the lactogenic hormone at the cellular level is in association with the mitochondria and microsomes of the secretory cells. Studies of the nature of this association of the lactogenic hormone with the mitochondria and microsomes would be expected to yield information basic to an understanding of the mode of action of the lactogenic hormone at the cellular level. To this end, studies of the chemical nature of this association have been carried out.

Due to the nucleoprotein and lipoprotein nature of the cellular particulates this investigation has been limited to study of the involvement of two chemical moieties, the pentosenucleic acid and the lipid, in the association of the lactogenic hormone with the mitochondria and microsomes. Demonstration of the involvement of particulate lipid or nucleic acid in the lactogenic hormone-particulate association would at least indicate something of the nature of this association and to that

* Contribution from the Department of Dairy Husbandry, Missouri Agri. Exp. Station, Journal Series No. 1565. Approved by the Director.

This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.