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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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

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extent add to the understanding of the basic biochemical mechanisms involved.

Methods and materials. The method which was used for the labeling of the lactogenic hormone with radioactive iodine has been described(1). Experimental treatment of the animals and preparation of the samples for counting of sample radioactivity were also described(1). For all studies reported here an isotonic sucrose homogenate of the mammary gland from pseudopregnant rabbits, which had been injected intraductally with radioactive iodine labeled lactogenic hormone was centrifuged at 1000 g for 10 minutes to remove nuclei and the supernatant from this centrifugation was used for fractionation. Grinnan and Mosher(2) used 4 M guanidine hydrochloride for the production of a highly polymerized and apparently undenatured RNA from liver. This same procedure has been used in this study for the precipitation of nucleoprotein from the mammary homogenate. A second procedure for precipitation of nucleic acids and nucleoproteins has been studied by Berkman, Housewright and Henry (3) and Euler and Heller(4). These studies indicated that streptomycin was effective in precipitation of cytoplasmic nucleoproteins. In the present work the homogenate, as prepared above, was brought to pH 7.0-7.4 and streptomycin added to a final concentration of M/200 to M/400, held at room temperature for one hour with occasional shaking, chilled to 0°C for one hour and the nucleoprotein precipitate removed. Two methods which have been used for the solubilization of particulate proteins were studied for their action on the lactogenic hormone-particulate association. Wainio *et al.*(5) studied the solubilizing effect of two to four percent desoxycholate on the cytochrome oxidase associated with the insoluble particulates of heart muscle preparation. Morton(6) studied the effect of excess n-butyl alcohol on the solubilization of enzymes associated with insoluble cytoplasmic particulates. The isotonic sucrose homogenate with the nuclei removed was treated for three hours at room temperature with these reagents, centrifuged at 25000 g for 90 minutes, dialyzed against distilled water and the streptomycin precipitable material counted as being the material taken into solution by these procedures. The results obtained with these methods are of interest due to the apparent dependence of these methods on the removal of lipids and the rupture of lipoprotein bonds.

TABLE I. Precipitation of Activity from Unfractionated Particulate Preparations.

4M guanidine HCl precipitation			Streptomycin precipitation		
Ppt., cpm	S'nat., cpm	% ppt.	Ppt., cpm	S'nat., cpm	% ppt.
243867	120070	67.0	476015	113406	80.7
71531	53388	57.2	206666	35995	85.1
76482	29284	72.3	111549	14886	88.2
206240	36873	84.8	82860	10702	88.5
601242	115590	83.8			
375056	137538	73.1			
1850	1556	54.3			
27806	4988	84.7			
152759	11921	92.7			
Avg % ppt.	74.4 ± 14.2			85.6 ± 10.3	

Results. The studies reported here for the distribution of radioactivity following precipitation of the nucleoprotein with guanidine hydrochloride or streptomycin indicate that the cytological localization of the lactogenic hormone noted previously is, in fact, an association of the lactogenic hormone with the nucleoprotein of the cytoplasm.

These data (Table I) indicate that precipitation with 4M guanidine hydrochloride precipitates approximately 75% of the lactogenic I^{131} hormone as nucleoprotein. Approximately 85% of the lactogenic I^{131} is precipitated with streptomycin. It is seen that the agreement between the two methods is rather good, with at least 75% of the radioiodine activity labeling the lactogenic hormone being precipitated as the nucleoprotein by these methods.

The experiments using desoxycholate or n-butyl alcohol in attempts to solubilize the labeled lactogenic hormone-particulate association have given essentially negative results (Table II). These data, while not extensive, indicate by their negative nature that the particulate lipids are probably not involved in the hormone-particulate association.

Discussion. Basic to an understanding of

TABLE II. Solubilization of Particulates as Measured by I^{131} Activity of Non-Dialyzable, Streptomycin Precipitable Material.

	4% desoxycholate	Excess n-butyl alcohol	
	No. 1	No. 1	No. 2
Cpm soluble	17760	9450	7953
Cpm unfractionated particulate preparation	423620	423620	292035
% soluble	4.2	2.2	2.7
% insoluble or dialyzed	95.8	97.8	97.3

the mode of action of a particular hormone will be information which indicates where and how the metabolism of the target cell is controlled by the hormone. These studies appear to implicate the pentosenucleoprotein of the cytoplasmic particulates as being important in the action of the lactogenic hormone on the secretory cells of the mammary gland. Little is known of the function of the nucleic acid and nucleoprotein of the cytoplasm but it has appeared that they are involved in the synthetic processes of the cell, particularly those in which the cytoplasmic particulates are involved. It may be postulated that the association of the lactogenic hormone with the cytoplasmic particulate nucleoprotein results in changing the conditions of enzyme attachment to the particulate nucleoprotein or in changing the activity of the enzymes which are already in association with the nucleoprotein. Such changed conditions of enzyme attachment, affecting the enzymatic composition or activity of the cytoplasmic particulates, could result in changing the direction of the synthetic activity of the cell as occurs with the initiation of lactation.

Summary. Studies have been carried out with lactogenic hormone labeled with radioactive iodine in pseudo-pregnant rabbit mammary gland which indicate that the labeled hormone becomes associated with the cytoplasmic particulate nucleoprotein. Other data suggests that the particulate lipids are probably not involved in the lactogenic hormone-particulate association. These findings have been discussed in terms of their relation to the shift in synthesis which occurs in the mammary gland with the initiation of lactation.

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Carbon and Water Contents of Mouse and Rat Tissues. (22090)

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In reporting tissue analyses for radiocarbon, tracer chemists have employed a wide variety of units (*e.g.* per cent of injected dose, specific activity of tissue carbon, total activity per organ, activity per unit weight of fresh or dried tissue, etc.). Sometimes the choice is clearly dictated by the immediate context of the measurement; at other times the units are those most conveniently derived from the chosen method of radioassay (*e.g.* specific activity when $BaC^{14}O_3$ is assayed at "infinite thickness"). In analyzing and comparing data from several published sources, the reader must frequently translate from one set of units to another. Adequate information, however, is seldom presented for even an approximate conversion.

Table I records soft-tissue concentrations

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