malfunction of the divisive process to a "parasitism" of the mitotic apparatus, the failure of colchicine to prevent development of aberrant chromatin configurations suggests that other factors, as yet undiscovered, are responsible for this nuclear pycnosis.

Summary.—Reovirus becomes closely associated with the mitotic apparatus of L cells. During virus development the spindle tubules become coated by a dense granular or fibrous material and are contiguous to aggregates of progeny virus particles. Abolition of spindle tubules by colchicine alters the site but not the rate of virus development, showing that the spindle is not obligatory for multiplication of reovirus.

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[1] Sabin, A. B., *Science*, **130**, 1387 (1959).
[2] Rhim, J. S., L. E. Jordan, and H. D. Mayor, *Virology*, **17**, 342 (1962).
[3] Gomatos, P. J., I. Tamm, S. Dales, and R. M. Franklin, *Virology*, **17**, 441 (1962).
[4] Franklin, R. M., *Proc. Soc. Exptl. Biol. Med.*, **107**, 651 (1961).
[5] Drouhet, V., *Ann. inst. Pasteur*, **98**, 618 (1960).
[6] Tournier, P., and M. Plissier, *Presse méd.*, **68**, 683 (1960).
[7] Dales, S., *J. Cell Biol.*, **13**, 303 (1962).
[8] Sabatini, D. D., K. Bensch, and R. J. Barrnett, *J. Cell Biol.*, **17**, 19 (1963).
[9] Dulbecco, R., and M. Vogt, *J. Exptl. Med.*, **99**, 167 (1954).
[10] Palade, G. E., *J. Exptl. Med.*, **95**, 285 (1952).
[11] Luft, J., *J. Biophys. Biochem. Cytol.*, **9**, 409 (1961).
[12] Karnovsky, M. J., *J. Biophys. Biochem. Cytol.*, **11**, 729 (1961).
[13] Mazia, D., and K. Dan, these PROCEEDINGS, **38**, 826 (1952).
[14] Dales, S., *Exptl. Cell Research*, **19**, 577 (1960).
[15] Bernhard, W., and E. de Harven, *Proc. IV Internat. Conf. Electron Microscopy* (Berlin: Springer-Verlag, 1958), vol. 2, p. 217 (1960).
[16] Harris, P., *J. Cell Biol.*, **14**, 475 (1962).
[17] Spendlove, R. S., E. H. Lennette, C. O. Knight, and J. N. Chin, *J. Immunol.*, **90**, 549 (1963).
[18] Spendlove, R. S., E. H. Lennette, and C. John, *J. Immunol.*, **90**, 554 (1963).

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*BIOSYNTHESIS OF THYROGLOBULIN: RELATIONSHIP TO RNA-TEMPLATE AND PRECURSOR PROTEIN**,†

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With the use of the antibiotic actinomycin, which inhibits cellular RNA synthesis[1–4] by binding with guanine residues on the DNA,[5] experiments on the physiological activity of the RNA-template for protein synthesis have become possible. Such studies in *B. subtilis* have been interpreted as providing evidence for the short life of the RNA template.[6] In several other systems, however, protein synthesis has been shown to continue in the absence of synthesis of RNA. Protein synthesis takes place in reticulocytes in the absence of RNA synthesis.[7–8]

Protein continues to be made for some time in mouse fibroblasts[1] and fertilized sea urchin eggs[9] after complete inhibition of RNA synthesis of actinomycin. This demonstration of the dissociation between protein and RNA formation has not been limited to animal tissues.[10, 11] These results have led to the concept that the protein-forming units vary considerably in their stability and that, especially in well-differentiated cells, such units may possess long lives.

The thyroid gland is composed of highly differentiated nucleated cells which produce thyroglobulin, a large protein readily isolated for direct study. It appeared likely that the RNA-template specifically concerned with thyroglobulin synthesis would possess considerable stability and that thyroglobulin formation would be resistant to the action of actinomycin. This report will present evidence in favor of this concept and also data on the involvement of a precursor protein in thyroglobulin biosynthesis.

Experimental.—Thyroid gland slices and incubation conditions: Fresh lamb thyroids were obtained from a local abattoir and immersed in iced phosphate-buffered saline within 20 min of slaughter. The glands were cleared of fat and connective tissue, sliced thin with a razor blade, and incubated in sterile Eagle's medium for suspension cultures[12] containing 0.1 mM of the non-essential amino acids, penicillin, and streptomycin. Unlabeled leucine was omitted from the original medium and added with or without its radioactive label at the desired specific activity. Incubation volumes of either 2.0 ml or 4.0 ml contained 2–4 slices of thyroid. Each experiment consisted of slices taken from one lobe of the same gland if possible; if not, an equal number of slices from each lobe were added to each flask. Before addition of the slices the flask was pre-incubated for 15 min at 37° in a Dubnoff metabolic shaker into which 100% O_2 was bubbled. Gentle shaking and oxygenation were continued for the duration of the experiment. On long incubations, contamination by microorganisms was ruled out by standard bacteriological techniques.

Preparation of thyroid RNA for sucrose gradient centrifugation: Following incubation, thyroid RNA was isolated by the method of Hiatt,[13] except that after treatment with DNAase and precipitation with 67% alcohol, the RNA was passed through a 1 × 7 cm G-25 Sephadex column, which removed all traces of phenol, oligodeoxyribonucleotides, and unincorporated labeled precursor. The effluent containing the RNA was reprecipitated with alcohol, taken up in 0.20 ml of 0.10 *M* NaCl and 0.01 *M* potassium acetate buffer pH 5.0, and layered on a 5–20% sucrose gradient containing the same salt and buffer concentrations.

Preparation of thyroid soluble protein for sucrose gradient centrifugation: Following incubation, the flask contents were ground in a Potter-Elvehjem glass homogenizer and centrifuged ten min at 27,000 × *g* at 1–2°C. The particulate fraction was extracted with TBS and assayed for radioactivity by the method of Wool and Krahl.[14] The protein in the supernatant fluid was precipitated by the addition of saturated ammonium sulfate at pH 5.0 or pH 6.9 to the desired concentration. The precipitate was separated by centrifugation and dissolved in 4.0 ml of TBS and reprecipitated two times with ammonium sulfate. The final precipitate was dissolved in TBS in the minimal volume that would float on 10% sucrose. A 0.2 ml aliquot was layered on the 10–25% sucrose gradient for centrifugation.

Sucrose gradient fractionation: Sucrose gradients were made by the method of Martin and Ames,[15] with 4.7 ml in each tube. Gradients for RNA measurements were 5–20% sucrose with 0.10 *M* NaCl and 0.01 *M* potassium acetate pH 5.0. Gradients for protein measurements were made with 10–25% sucrose in TBS. The Spinco model L preparative ultracentrifuge rotor SW 39 was used for centrifugation at 39,000 × *g*. Protein samples were centrifuged for 9 hr. Ten drop fractions were collected in all experiments, each tube was diluted with 1.0 ml water, the optical density was measured at 260 mμ for RNA and 280 mμ for protein, and an aliquot added to 15 ml of Bray's solution.[16] The samples were counted in a Packard Tri-Carb liquid scintillation spectrometer.

Thyroglobulin antibodies: Anti-sheep thyroglobulin antibodies were prepared using rabbits with sheep thyroglobulin purified by the procedure of Derrien, Michel, and Roche.[17] Sucrose

gradient fractions were precipitated with antibody after 3 mg of carrier sheep thyroglobulin had been added. The precipitates were dissolved in 1.0 ml Hyamine 10 × and counted in the scintillation spectrometer. The counting fluid contained 0.4% PPO and 0.01% POPOP in 20 ml of toluene.

Thyroid autoradiograms: Incubated slices were fixed in 10% buffered formalin, embedded, and 4 micron slices placed on slides. Kodak AR-10 stripping film was used. Exposure time was six weeks.

Chemicals: H^3-thymidine (6.05 c/mmole), H^3-uridine (1.0 c/mmole), C^{14}-uridine (125 μc/mg), and C^{14}-L-leucine (850 μc/mg) were obtained from Schwarz BioResearch, Inc. H^3-L-leucine-4, 5 T (7.1 c/mmole) was from Nuclear-Chicago Corporation. Actinomycin C_1 (D) was a gift of Dr. E. Alpert of Merck, Sharp and Dohme Research Laboratories. Carrier-free NaI^{125} and Pi^{32} were from Volk Radiochemical Company. Puromycin was obtained from Nutritional Biochemicals Corporation. Sephadex G25 and G200 were from Pharmacia Fine Chemicals, Inc.

Results and Comments—Formation of a rapidly labeled RNA in thyroid slices and inhibition of RNA synthesis with actinomycin: As has been shown for actively multiplying mammalian cells in culture[18–20] and regenerating rat liver,[13] thyroid slices incubated for 15 min with H^3-uridine or Pi^{32} incorporate these labeled materials into RNA with a sedimentation constant of about 44S (Fig. 1*A*). Similarly, after longer exposures the label corresponds in position with the 30, 18, and 4S optical density peaks (Fig. 1*B*). Autoradiographic studies with H^3-uridine and H^3-thymidine show that the observed RNA synthesis is associated with the follicular cells of the thyroid slice and that DNA labeling is a rare event. RNA synthesis therefore is a property of cells known to be involved in thyroglobulin synthesis and is not limited to a small population of undifferentiated cells undergoing cell division. It appeared therefore that, in this highly differentiated organ composed primarily of nondividing cells, a rapidly labeled RNA fraction is formed which with time contributes to the labeling of microsomal RNA. In the presence of actinomycin (10 γ/ml) all labeling of RNA is abolished except for a small amount associated with the 4S fraction. As seen in Fig. 2*A*, the addition of actinomycin leads to rapid inhibition of uridine incorporation into the total RNA, and this is, in fact, associated with a significant loss of label from previously synthesized RNA. Upon prolonged preincubation with 10 γ/ml of actinomycin, RNA synthesis becomes barely detectable. Two hr of preincubation with 10 γ/ml of actinomycin results in less than 1% of the RNA synthesis found in the control.

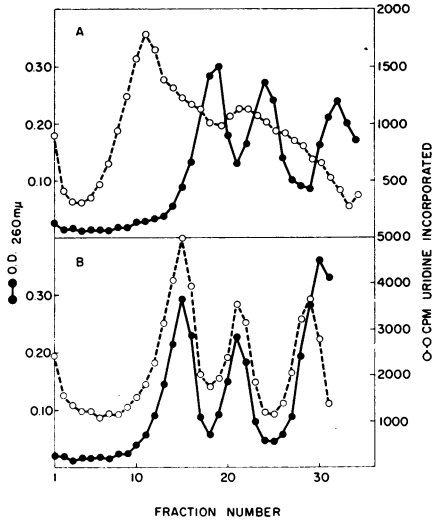

FIG. 1.—Gradient centrifugation of thyroid RNA labeled with H^3-uridine. Slices were incubated (*A*) 15 min in 2 ml of medium with 100 μc of H^3-uridine, and (*B*) 8 hr in 4 ml of medium with 200 μc of H^3-uridine and 2.0 μmoles of unlabeled uridine. RNA was prepared for sedimentation analysis as in *Experimental*. (*A*) was centrifuged 2¾ hr and (*B*) 3 hr.

Effect of actinomycin on the synthesis of thyroglobulin and other proteins: Despite the abrupt shutoff in RNA synthesis due to actinomycin, the incorporation of

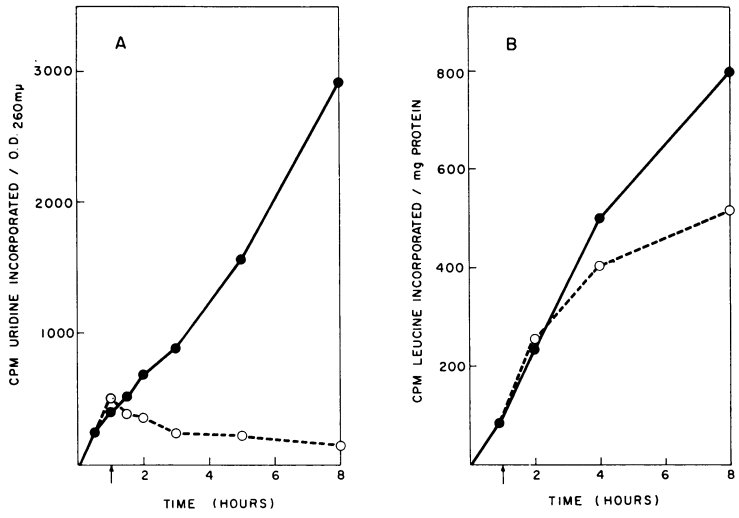

FIG. 2.—Effect of actinomycin on RNA and protein synthesis in thyroid slices. (*A*) Slices were incubated in 4 ml of medium with 40 μc of H^3-uridine. At the indicated times the slices were removed, blotted, and homogenized in cold 5% TCA. RNA was prepared for analysis as per Reich *et al.*[1] An aliquot was counted in Bray's solution as described. (*B*) Slices were incubated in 4 ml of medium containing 2.0 μmoles of uridine and 4 μc of C^{14}-L-leucine. Incubation was stopped as in (*A*). Protein was worked up as per Wool and Krahl.[14] ●—● represents the control, O - -O represents incubations in which actinomycin (10 γ/ml) was added at 1 hr.

leucine into total cell protein continues at the control rate for about one hr and at a reduced but measurable rate for the subsequent 6 hr (Fig. 2*B*). In other experiments leucine incorporation into protein continued at almost the 6-hr rate for at least another 15 hr. The data suggested that the synthesis of some protein fraction was relatively sensitive to the action of actinomycin and linked to that of RNA, whereas some other was independent of the formation of new RNA. Support for this was obtained by studying the effect of actinomycin on the incoporation of leucine into separate fractions of the total cellular protein. A saline extract of incubated thyroid slices was fractionated by centrifugation at 27,000 × *g* into a nonextractable particulate fraction and a soluble fraction; the latter was divided by ammonium sulfate precipitation into 0–35, 35–45, and >45 per cent fractions. Whereas the incorporation of leucine into the proteins of the particulate fraction and the 0–35% ammonium sulfate fraction were sensitive to actinomycin, incorporation into the 35–45% ammonium sulfate fraction which contains the thyroglobulin was relatively resistant to actinomycin. This suggested that the synthesis of the thyroglobulin *per se* would possess considerable resistance to the action of actinomycin.

Following repeated ammonium sulfate precipitations (0–50 or 0–70 per cent) of the saline extract of incubated thyroid slices to remove nonprotein label, the 19S thyroglobulin was separated from other proteins by means of the sucrose gradient centrifugation technique. After incubation of slices with labeled leucine or I^{125} (Fig. 3), the optical density peak at 280 mμ corresponding to the 19S position was labeled with the isotope. The labeled thyroglobulin was precipitable with

anti-sheep thyroglobulin antibody and, as expected from its molecular size, appeared early in the eluate from a Sephadex G200 column. The higher specific activity of I^{125} on the lighter side of the 19S material (Fig. 3) was also seen with radioactive leucine and may be related to the heterogeneity of thyroglobulin.[21, 22] Whereas most of the I^{125} is found to be associated with the 19S protein peak, when leucine is used as the label a large proportion of the radioactivity is associated with the 3–8S region. This is especially true when the 50–70 per cent ammonium sulfate fraction is included on the sucrose gradient centrifugation. Ammonium sulfate fractionation of the saline extract showed the 3–8S radioactivity to be comprised of material precipitating both below 35 and above 45 per cent ammonium sulfate (the latter may be related to the thyralbumin fraction described by Shulman *et al.*).[23] The incorporation of radioactive leucine into the proteins of the 3–8S fraction was considerably more sensitive to the action of actinomycin than that into thyroglobulin.

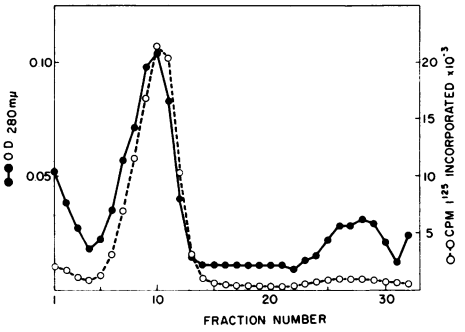

FIG. 3.—Labeling of thyroglobulin with I^{125} by thyroid slices. Slices were incubated 200 min in 4 ml of medium with 0.4 μmole of L-leucine and 10 μc of I^{125}. Soluble protein precipitated by 70% ammonium sulfate was prepared for sedimentation analysis as in *Experimental*.

Even after preincubation with high levels of actinomycin for 5, 10, 15, and 21 hr, the labeling of thyroglobulin over the subsequent 5 hr (followed by sucrose gradient centrifugation) was not significantly different from the control which was preincubated the same length of time without actinomycin. After the first 5 hr of incubation, the rate of leucine incorporation into 19S material remains relatively constant for the next 20 hr or so. The result of a 21-hr preincubation experiment is shown in Fig. 4 (see also Figs. 6*C* and 6*D*). It can be seen, furthermore, that a 3–8S protein fraction of high specific activity is present in the 35–45 per cent ammonium sulfate fraction. This fraction whose synthesis is inhibited to a large extent by preincubation with actinomycin may be removed to a considerable degree from thyroglobulin by closer fractionation with ammonium sulfate. This probably represents contamination by proteins appearing predominantly in the 0–35 or 45–70 per cent ammonium sulfate frac-

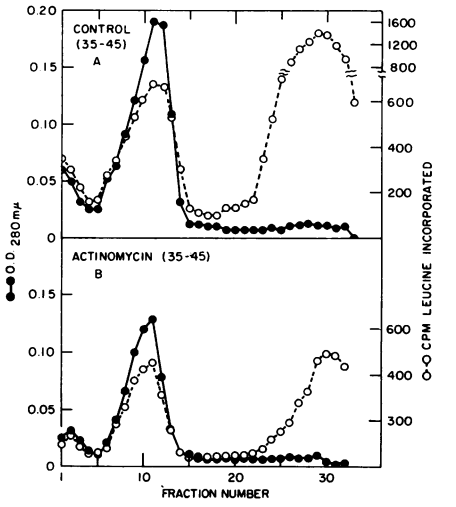

FIG. 4.—Resistance of thyroglobulin labeling to preincubation with actinomycin. Thyroid slices were incubated for 21 hr in 4 ml of medium containing 0.4 μmole of L-leucine (*A*) without and (*B*) with 10 γ/ml actinomycin. H^3-L-leucine (100 μc) was then added and incubation continued for 5 hr. A 35–45% ammonium sulfate fraction was prepared for sedimentation analysis as in *Experimental*.

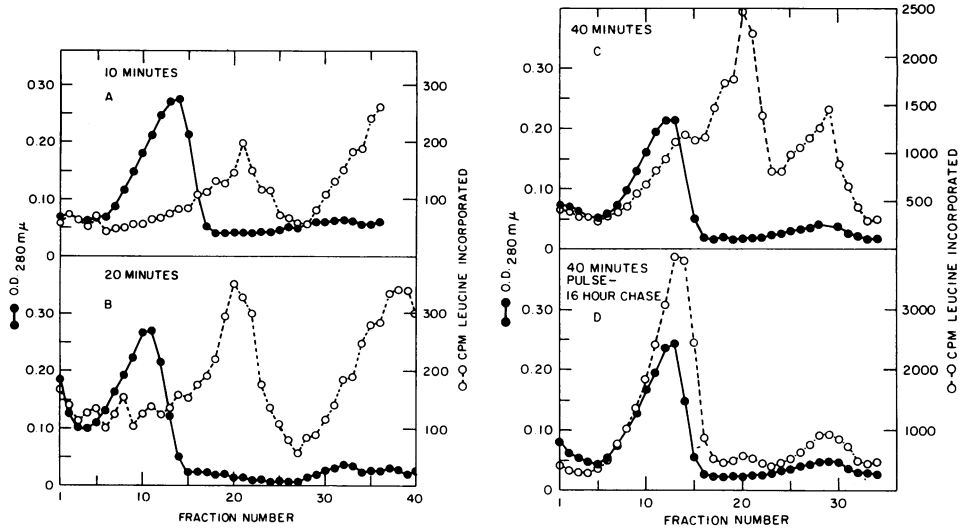

FIG. 5.—Gradient centrifugation of thyroid protein pulse-labeled with H^3-leucine. Slices were incubated in 4 ml of medium containing 200 μc of H^3-leucine and removed at (*A*) 10, (*B*) 20, (*C*) 40, and (*D*) 40 min. In (*D*) the slices were then blotted, rinsed in cold phosphate-buffered saline, and transferred to 4 ml of fresh medium containing 4 μmoles/ml of unlabeled leucine. Incubation was continued for an additional 16 hr. In (*A–C*) slice protein was used, whereas in (*D*) the entire flask content was used. The soluble protein precipitated by 50% ammonium sulfate was prepared for sedimentation analysis as in *Experimental*.

tions, both of these fractions being sensitive to actinomycin. In contrast with actinomycin, puromycin rapidly inhibits labeling of thyroglobulin with leucine.

Evidence for a thyroglobulin precursor protein: Whereas most of the radioactive leucine was associated with 19S or 3–8S material on prolonged incubations, short periods of exposure to labeled amino acid showed a discrete peak of radioactivity in the 12S region as well as radioactivity in the lighter material (Figs. 5*A*, 5*B*, and 5*C*). With time (Figs. 5*B* and 5*C*), radioactivity appeared to move into the 19S region. Figure 5*D* shows that following a 40-min pulse, a 16-hr chase with unlabeled leucine resulted in the radioactivity becoming associated mainly with the 19S region. The label which was in the 3–8S area has changed little, whereas that in the 12S area has decreased considerably. These experiments are consistent with the thesis that the 12S substance is converted into 19S material. It should be noted that very short pulses with I^{125} do lead to a slight peak of label in the 12S region, but, even at 2 min, I^{125} is associated to a greater extent with thyroglobulin; in addition, there is some labeling of the lighter region. Longer incubations in the presence of radioactive leucine (Figs. 6*A*, 6*B*, and 6*C*) show a progression of radioactivity into the 19S thyroglobulin after that in the 12S fraction has reached a plateau.

Preliminary experiments with puromycin suggest that the 12S material can be converted into 19S in the absence of further protein synthesis. This is compatible with the 12S material's being a subunit precursor of thyroglobulin which does not require additional soluble RNA-ribosome interaction for conversion into thyroglobulin. By analogy with the thyroglobulin dissociation studies of Edelhoch,[25] the radioactive 12S unit formed by the thyroid slices may be half the size of the thyroglobulin molecule.

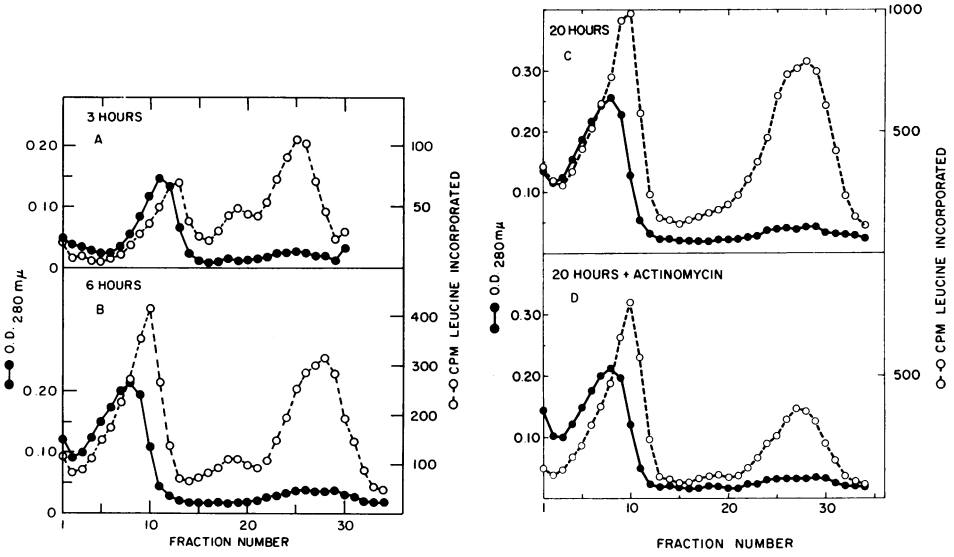

FIG. 6.—Time course of labeling of thyroid protein with C^{14}-leucine. Thyroid slices were incubated in 4 ml of medium with 0.4 μmole of L-leucine and 1.0 μc of C^{14}-L-leucine. In (*D*) 10 γ/ml of actinomycin were added at 1 hr. Protein preparation and sedimentation analysis were as in Fig. 5. The apparent small decrease in thyroglobulin specific activity due to actinomycin is actually caused by the elevated radioactivity baseline in the control incubation. This radioactivity which is inhibited by actinomycin precipitates mainly below 35% ammonium sulfate and thus was removed by the fractionation used in Fig. 4 prior to layering on the sucrose gradient.

The experiments presented here dealt primarily with the 0–50% ammonium sulfate fraction of thyroid slice extract. The 50–70 per cent fraction (3–8S material) is even more highly labeled and may contain precursor material for the 12S protein. These fractions are heterogeneous and probably contain both actinomycin-sensitive and -insensitive proteins.

Summary—Thyroglobulin formation by thyroid slices has been shown with the use of actinomycin to be independent of RNA synthesis. By the sucrose gradient centrifugation technique and pulse labeling with radioactive leucine, evidence was found for a 12S precursor protein in thyroglobulin synthesis.

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† The following abbreviations are used: TCA, trichloroacetic acid; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2-(5-phenyloxazoyl)-benzene; Pi, inorganic orthophosphate; TBS, Tris buffered saline containing 0.9% NaCl and 0.01 *M* Tris, pH 7.1.

[1] Reich, E., R. Franklin, A. Shatkin, and E. Tatum, these PROCEEDINGS, **48**, 1238 (1962).

[2] Goldberg, I. H., and M. Rabinowitz, *Science*, **136**, 315 (1962).

[3] Hurwitz, J., J. J. Furth, M. Malamy, and M. Alexander, these PROCEEDINGS, **48**, 1222 (1962).

[4] Reich, E., I. H. Goldberg, and M. Rabinowitz, *Nature*, **196**, 743 (1962).

[5] Goldberg, I. H., M. Rabinowitz, and E. Reich, these PROCEEDINGS, **48**, 2094 (1962), and **49**, 226 (1963).

[6] Levinthal, C., A. Keynan, and A. Higa, these PROCEEDINGS, **48**, 1631 (1962).

[7] Marks, P. A., E. R. Burka, and D. Schlessinger, these PROCEEDINGS, **48**, 2163 (1962).

[8] Reich, E. G., B. Mach, and E. L. Tatum, in *Symposium on Informational Macromolecules*, Rutgers University, in press.

[9] Gross, P. R., and G. H. Cousineau, *Biochem. Biophys. Res. Comm.*, **10**, 321 (1963).

[10] del Valle, M. R., and A. I. Aronson, *Biochem. Biophys. Res. Comm.*, **9**, 421 (1962).
[11] Gause, G. F., and A. V. Laiko, *Nature*, **196**, 167 (1962).
[12] Eagle, H., *Science*, **130**, 432 (1959).
[13] Hiatt, H. H., *J. Mol. Biol.*, **5**, 217 (1962).
[14] Wool, I. G., and M. E. Krahl, *Am. J. Physiol.*, **196**, 961 (1959).
[15] Martin, R. G., and B. N. Ames, *J. Biol. Chem.*, **236**, 1372 (1961).
[16] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
[17] Derrien, Y., R. Michel, and J. Roche, *Biochim. et. Biophys. Acta*, **2**, 454 (1948).
[18] Scherrer, K., and J. E. Darnell, *Biochem. Biophys. Res. Comm.*, **7**, 486 (1962).
[19] Tamaoki, T., and G. C. Mueller, *Biochem. Biophys. Res. Comm.*, **9**, 451 (1962).
[20] Perry, R. P., these PROCEEDINGS, **48**, 2179 (1962).
[21] Ingbar, S. H., B. A. Askonas, and T. S. Work, *Endocrinology*, **64**, 110 (1959).
[22] Robbins, J., *J. Biol. Chem.*, **238**, 182 (1963).
[23] Shulman, S., N. R. Rose, and E. Witebsky, *Fed. Proc.*, **16**, 433 (1957).
[24] On prolonged preincubation (greater than 5 hr) with 10λ/ml of actinomycin, some variability in the degree of the inhibition of thyroglobulin labeling has been observed which may be related to the previous biological history of a thyroid gland. It has been found, however, that following a 5-hr preincubation with actinomycin, thyroglobulin labeling over the ensuing 5 hr is consistently 70–96% of the control. These experiments will be detailed elsewhere.
[25] Edelhoch, H., *J. Biol. Chem.*, **235**, 1326 (1960).

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*THE STRUCTURE OF CARBOXYPEPTIDASE A, I. A TWO-DIMENSIONAL SUPERPOSITION FUNCTION**

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Since bovine pancreatic carboxypeptidase A is a well-characterized enzyme of relatively low molecular weight (34,300),[1] is readily crystallized, and complexes an exchangeable metal ion at its active site,[2] it seems eminently suitable for structure analysis by the techniques of X-ray crystallography. A preliminary examination of crystals prepared by the procedure of Allan[3] was made by Kraut,[4] who concluded that the space group is $P2_1$ with one molecule per asymmetric unit. He reported cell dimensions of the zinc-containing enzyme as $a = 51.7$ Å, $b = 60.1$ Å, $c = 47.1$ Å, and $\beta = 97° 25'$, which we confirm.

Experimental.—Suspensions of similarly prepared carboxypeptidase A crystals (CPD-Zn), kindly supplied to us by Professor H. Neurath, were used in attempts to prepare isomorphous derivatives. Aliquots of the crystal suspensions were allowed to dialyze against LiCl-barbital buffer containing lead citrate. After several weeks, photographs showed intensity changes relative to the parent crystals. The cell dimensions of the derivative were found to be: $a = 51.5$ Å, $b = 59.9$ Å, $c = 47.2$ Å, $\beta = 97° 30'$. These values suggest that the lead derivative is isomorphous with the parent enzyme.

Measurement of the lead derivative: Precession photographs were taken of the (*h*0*l*) zones of the zinc enzyme and the lead derivative, and the intensities were measured photometrically on a Joyce-Loebl microdensitometer. After the standard Lorentz and polarization corrections were applied, the relative scale factor was found, and a difference Patterson map, with coefficients $(|F_{Pb}| - |F_{Zn}|)^2$, was computed (Fig. 1).