mission Loyalty Review Board. However, even if the Loyalty Review Board issues a favorable decision, the department may still consider the employee to be a security risk.

Applications for jobs in these three departments are handled by the Civil Service Commission and so loyalty clearance is required, as in the case of other government departments and agencies described before. Once employed, however, a person may require additional security clearance under the procedures just stated.

Army-Navy-Air Force Contracts

The Army, with the concurrence of the Navy and Air Force, has set up security clearance procedures for employees of contractors of the Army, Navy, and Air Force (*4*). Designated military officials may grant clearance directly, but doubtful cases are referred to the Army-Navy-Air Force Personnel Security Board, which may direct that the employee be discharged or suspended. This board holds no hearings. Any action may be appealed by the person involved to the Industrial Employment Review Board, in the office of the Provost Marshall General. A hearing before this board is possible only in Washington, D. C. The board consists of representatives, generally military men, from each of the three departments. No provision is made for the presentation of charges to the individual. The entire hearing is classified and no records or notes on the hearing may be kept by the individual or his lawyer. No further appeals are possible.

All of the procedures that have been described here differ from ordinary legal procedure in this country in at least four major respects (*6*): (1) no separation between the executive and judicial responsibilities is made in the processing of any loyalty or security cases, (2) no provision is made for a hearing board to write a specific finding of fact after a hearing, (3) confrontation and cross-examination of adverse witnesses are rarely permitted, and (4) the hearings are always closed to the public.

The procedural safeguards, the rights and privileges of individuals, are of particular interest to those scientists who may be involved in clearance problems. Thus, the chief items of the various procedures are summarized in Table 1. Such a condensation clearly cannot be used as the sole basis for judging and comparing various procedures, but may be useful as an index for further inquiry.

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Biological Synthesis of Radioactive Silk[1]

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FIBROIN, THE PROTEIN OF SILK, has been a classical object of study in attempts to elucidate the structure of proteins because it is stable and easily isolated and its composition is constant. Numerous and detailed analyses of its component amino acids (*1–3*) have revealed that glycine and alanine are present in exceptional concentrations, accounting, in fibroin hydrolyzates, for about three-quarters of the total number of amino acid residues and for about two-thirds of the total weight of the residues (*1, 2*). These unusual properties of fibroin, and the availability of C^{14}-labeled glycine and alanine

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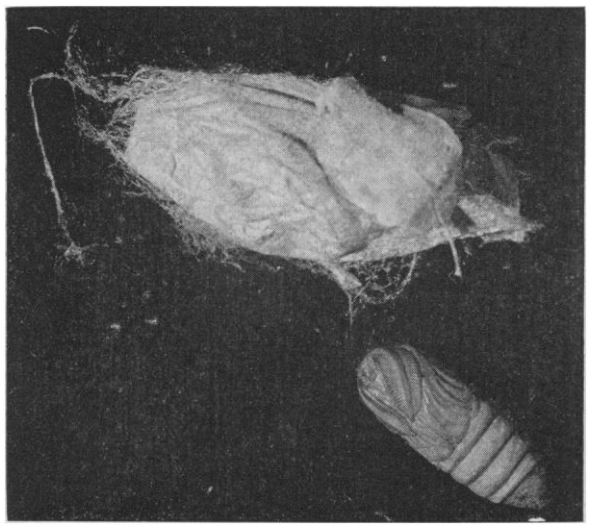

FIG. 1. Cecropia cocoon with a pupal stage of the insect removed therefrom.

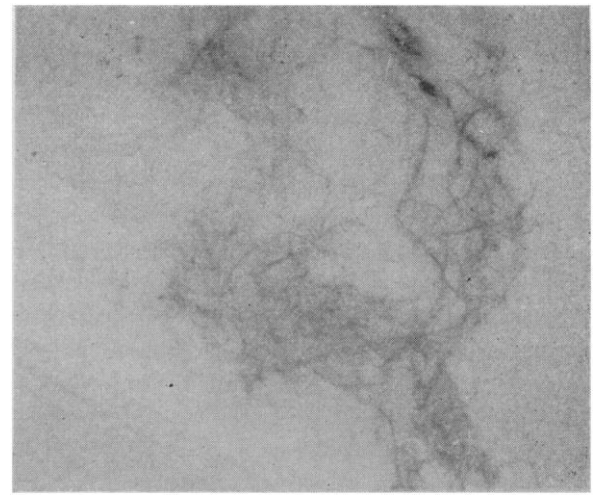

FIG. 3. Radioautograph of silk fibers teased from a radioactive cocoon (mg. 11 ×).

(*4, 5*), suggest a possible approach to the biological synthesis of a stable, homogeneous, radioactive protein.

As a first step in this direction we have tested the ability of the giant silkworm, *Platysamia cecropia,* to incorporate radioactive glycine and alanine into the silk it synthesizes. To this end, 0.05 cc of a mixture of radioactive glycine and alanine was injected into the body cavity of a mature silkworm (animal A). A total of 27,000 counts per minute was injected, as measured by an end window Geiger-Müller counter with a counting efficiency of about 8 percent under conditions described previously (*8*). The injection contained 0.013 mg of C^{14}-carboxyl-labeled glycine with an activity of 18,000 cpm and 0.02 mg of C^{14}-carboxyl-labeled DL-alanine, with an activity of 9,000 cpm.

Following the injection, the silkworm continued to feed normally for 24 hours. It then began spinning the cocoon shown in Fig. 1. A thin piece of cocoon fabric, removed and placed under the Geiger-Müller counter, exhibited radioactivity. A similar result was obtained with a silkworm (animal B) injected with 0.05 cc of a solution containing 18,000 cpm in 0.04 mg of C^{14}-carboxyl-labeled DL-alanine alone.

Fragments of cocoon from each insect were placed against unexposed film (Du Pont dental film No. 552-1 and No. 552-2 [cf. *6*].) and stored for periods of one to four weeks in the dark. The films were then developed for seven minutes at 68° F. Invariably, radioautographs which revealed details of the pattern of the silk fibers were obtained (cf. Figs. 2 and 3).

In order to test whether the radioactivity of the silk was actually present in the component amino acids, a fragment of cocoon A (73 mg) was cut into small pieces and homogenized into tiny fragments in water. The suspension was precipitated in 10-percent trichloroacetic acid, washed with trichloroacetic acid, and hydrolyzed overnight in an autoclave with the aid of 6 N hydrochloric acid. The ninhydrin procedure (*7*) was then performed on the protein hydrolyzate. Duplicate counts of the barium carbonate precipitate revealed 160 and 150 cpm per 0.1 mM $BaCO_3$ (*8*). Thus the radioactivity was shown to be present in the α-carboxyl group of some amino acid associated with the silk fibers.

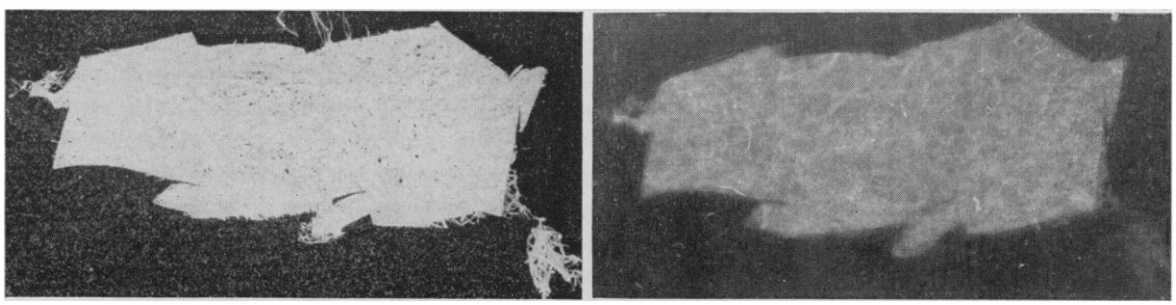

FIG. 2. a) Fragment of cocoon (mg. 3.5 ×). b) Radioautograph of the same fragment.

The possibility remained, however, that the labeled amino acids might be adsorbed onto the silk fibers, rather than incorporated into the peptide chain structure of the protein itself. Efforts were therefore made to dissolve the fibroin and determine whether or not the reprecipitated protein was still radioactive. There was not, unfortunately, a sufficient quantity of labeled silk to carry out such a procedure. Accordingly, 0.1 g of the labeled cocoon (animal A) was added to 0.9 g of inert silk, obtained from other cocoons, and the mixture was degummed by boiling with soap. The dried, degummed fibroin was then cut into tiny pieces with scissors and added to 43 cc of copper hydroxide-ethylene diamine solution [6 g $Cu(OH)_2$ and 3 g ethylene diamine in 100 cc of aqueous solution] (*2*). After ten minutes the silk had been partially dissolved. The suspension was then neutralized with 1.25 N acetic acid to pH 8, and filtered with suction. The clear, deep blue filtrate, containing dissolved silk, was added slowly to six volumes of acetone with stirring. The resulting precipitate was collected on a small Büchner funnel and washed with acetone and ether. The flaky, pale purple precipitate was suspended in 15 cc of water; the water turned a dark blue. The suspension was filtered onto a small Büchner funnel, and the light brown precipitate was washed with water and dried in an oven. Thirteen mg of this reprecipitated silk fibroin was hydrolyzed overnight in 1 cc of 6 N HCl in an autoclave. A ninhydrin determination was performed on the fibroin hydrolyzate, the evolved carbon dioxide being collected as barium carbonate and counted in the usual manner (*8*). There were 12.7 cpm per 0.1 mM barium carbonate when corrected to 0.1 mM thickness (5.4 mg per sq cm with our apparatus). Taking into account the tenfold dilution of the original labeled silk with unlabeled silk, there were 127 cpm per 0.1 mM of hydrolyzate of labeled silk fibroin.

As a result of these findings, we were encouraged to try an experiment *in vitro* on protein synthesis in the isolated silk gland of the insect. Two silkworms, in the final instar, were dissected and the four silk glands removed. These glands were incubated in a Warburg flask in 1.1 cc of Krebs-Ringer-phosphate medium of pH 7.4, which contained in addition 0.04 M sodium pyruvate and 0.37 mg of C^{14}-carboxyl-labeled glycine with an activity of 10,000 cpm. After 3½ hours' incubation at 37° C in an atmosphere of oxygen, the experiment was terminated. The glands were homogenized and washed, and the proteins were precipitated, hydrolyzed, and assayed for the presence of radioactivity. A total of 74 cpm was found in the 0.067 mM sample of $BaCO_3$, or 93 cpm per 0.1 mM of $BaCO_3$ as corrected to standard conditions. This experiment implies that the amino acids were incorporated into protein in the living silkworm gland *in vitro*. Since the fibroin was not isolated, there is no certainty, however, that silk rather than some other protein was synthesized under the conditions of the experiment.

The term "protein synthesis" is used in a broad sense. There remains the possibility that the labeled amino acids may have entered preformed fibroin molecules by "exchange" rather than by participation in a true synthesis of the fibroin molecule *de novo*. There may also be formation of side chain peptide bonds between the labeled amino acids and either amino or carboxyl groups not in the alpha position. These several processes may have conceivably all taken place under the experimental conditions.

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