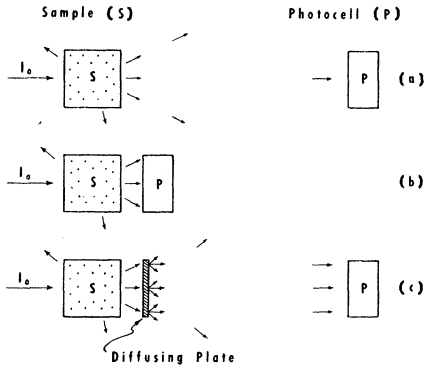

Fig. 1. Schematic diagrams of part of optical system of spectrophotometer.

grees. This arrangement gives an absorption spectrum which is the one most severely influenced by scattering. Arrangement *b* permits the photocell to collect light scattered angles up to about 30 to 50 degrees. Arrangement *c*, suggested by Shibata *et al.* (*3*), is equivalent to *b* with respect to the collection of scattered light and is experimentally more convenient. A diffusing plate, inserted behind the sample, causes a small but representative part of the light striking it to enter the photocell. Arrangement *c*, with identical diffusing plates made of opal glass placed behind the sample and the blank vessel, was used to minimize the influence of scattering.

The only spectrophotometer settings which were different for the two arrangements were those for the amplification of the photocell response and the band halfwidth (5 mμ for *a* and 7 mμ for *c* at a wavelength of 690 mμ; less at shorter wavelengths). Controls indicated that these differences did not influence the results. As a further check of the instrument and method, absorption spectra of chlorophyll in acetone were measured

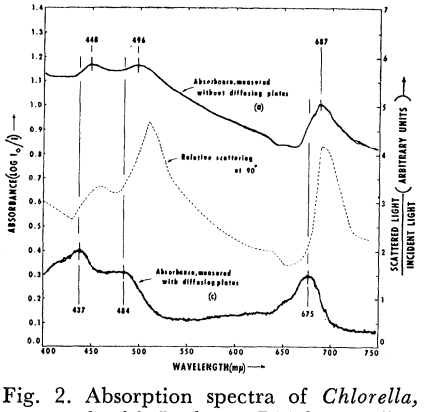

Fig. 2. Absorption spectra of *Chlorella*, measured with Beckman DK-2 recording spectrophotometer; arrangements *a* and *c* (shown in Fig. 1) were used. Both absorption curves are reproductions of original spectrophotometer recordings, each of which was made in triplicate. Center curve: scattering by *Chlorella* at 90 degrees. [From P. Latimer and E. Rabinowitch (*1*)]

with arrangements *a* and *c*. The shapes and positions of the bands on the two curves were identical, but the absorption between the bands differed slightly, probably because of multiple reflections between the diffusing plate and the sample vessel.

Figure 2 shows the absorption curves of a *Chlorella* suspension (10^{-3} ml of cells per milliliter) obtained with arrangements *a* and *c* and also the previously reported scattering curve of this organism. The latter curve is for light scattered at 90 degrees but is qualitatively representative of light scattered at most angles.

Of particular interest are the differences of more than 10 mμ between the positions of the major bands on the two absorption curves. Similar differences are found in the absorption curves published by Shibata *et al.* (*3*); however, these authors emphasized only the sharpness of the absorption bands as measured with diffusing plates and did not draw attention to the differences in the positions of the peaks.

Curve *c* is in reasonably good agreement with other absorption spectra reported for *Chlorella* (*1, 3, 4*); presumably the positions and shapes of the bands on it are approximately correct. The bands on curve *a*, however, seem to represent the *sum* of the scattering bands and the absorption bands (on curve *c*) of *Chlorella* which occur at different wavelengths. Light interference effects, such as those observed by Lothian and Lewis (*5*) in similar studies of red blood cells, may also have contributed to the differences between our "absorption" curves. Neither mechanism by itself can explain all related experimental results that have been reported to date. However, the asymmetry of the distorted absorption bands on curve *a* about the actual absorption maxima on *c* indicates that anomalous dispersion, which is also asymmetrical about absorption bands and which causes selective scattering, is of primary importance.

We are investigating the effects of scattering with a special spectrophotometer that allows the making of measurements of light transmitted or scattered at different angles. Some of the results, which confirm those shown in Fig. 2, should be useful in interpreting this distortion phenomenon.

Chlorella cells of the same strain but from other cultures gave quantitatively different results. While the red absorption maximum of all cells occurred at 675 mμ on the *c* curves, the position of this maximum on the *a* curves varied, with the culture, from 683 mμ to 691 mμ. The bands at the longest wavelengths were obtained with the cells which appeared to be in the rapid growth phase.

We also measured pairs of absorption curves for suspensions of chloroplasts and very small chloroplast fragments (*6*). The chloroplasts (average dimension 5 to 6 μ) gave a curve like some of those of *Chlorella*. The suspension of small fragments, however, behaved more like chlorophyll in solution; its bands on curves *a* and *c* were identical in shape and position.

The optical effect described here can significantly distort the positions and shapes of absorption bands of cells and their components. Some published absorption spectra of cells may show the influence of this effect. The influence would vary with the optical system of the spectrophotometer and with the scattering characteristics of the particular suspension.

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6 November 1957

Effect of Reserpine and Promazine on Diphosphopyridine Nucleotide Synthesis in Liver

It has been found by Kaplan *et al.* that the injection of nicotinamide into mice results in large increases of the levels of diphosphopyridine nucleotide (DPN) in tissue (*1*). The administration of "tranquilizing" agents, such as reserpine (*2*) and promazine (*3*), prior to the injection of nicotinamide results in the level of DPN in the liver being maintained at an elevated concentration for a prolonged period of time. This report (*4*) presents details of this observation and discusses some of their implications.

Hybrid male mice [(BALB/c An × DBA/2J)F_1, 8 to 10 weeks old, of 20 to 25 g body weight] were divided randomly into groups. Reserpine was administered subcutaneously 4 hours prior to the intraperitoneal injection of nicotinamide. Animals were sacrificed by cervical fracture at different time in-

tervals following administration of the nicotinamide. The livers were removed rapidly, weighed, and homogenized in cold 5 percent trichloroacetic acid. Diphosphopyridine nucleotide analyses were performed, in accordance with a modification (*5*) of the procedure of Levitas *et al.* (*6*). Four control groups were prepared as follows: by administering (i) reserpine only, (ii) nicotinamide only, and (iii) vehicle (*7*), without reserpine, plus nicotinamide and (iv) by using untreated animals.

Typical data from these experiments are presented in Fig. 1. The line labeled "untreated control" represents the DPN concentration in micrograms per gram of tissue (wet weight) for categories iv and i (untreated mice and mice that had received reserpine alone). Administration of reserpine alone did not produce a detectable change of DPN levels in the liver when comparison was made with the untreated controls. The curve labeled "no reserpine" represents data obtained from the mice that had been given nicotinamide only or that had been given a vehicle (for reserpine) plus nicotinamide. As has been shown, the administration of 500 mg of nicotinamide per kilogram of body weight results in an increase in DPN concentration in the liver. The data presented show an increase from about 500 μg to almost 4000 μg per gram in the first 12-hour interval after administration. In all the animals examined, 24 hours after the injection of nicotinamide alone, the DPN level had returned to the basal level and remained constant over the next 24-hour interval. When reserpine was administered before injection of the nicotinamide, the rate of accumulation of DPN was slower, but the same peak concentration was observed. It may be seen (Fig. 1, curve labeled "reserpine 10 mg/kg") that the high DPN level was maintained over a considerable length of time, being twice the basal level even 48 hours after administration of the nicotinamide.

A dose-response relationship may be observed by comparing the curves representing data obtained from animals that had been pretreated with injections of reserpine of 10, 4, or 1.6 mg per kilogram and from those that had been given no reserpine. Similar results have been obtained with other compounds possessing "tranquilizing" properties, such as deserpidine (*2*), promazine, and chlorpromazine. Compounds related to reserpine and promazine which are not "tranquilizing" agents failed to maintain the elevated DPN level. Meprobamate, pentobarbital, and ethanol were ineffective, even though the doses used kept the animals in a comatose state. It is interesting to note that the amount of the chlorpromazine required to produce this effect on DPN synthesis was about 20 times the dose of reserpine administered.

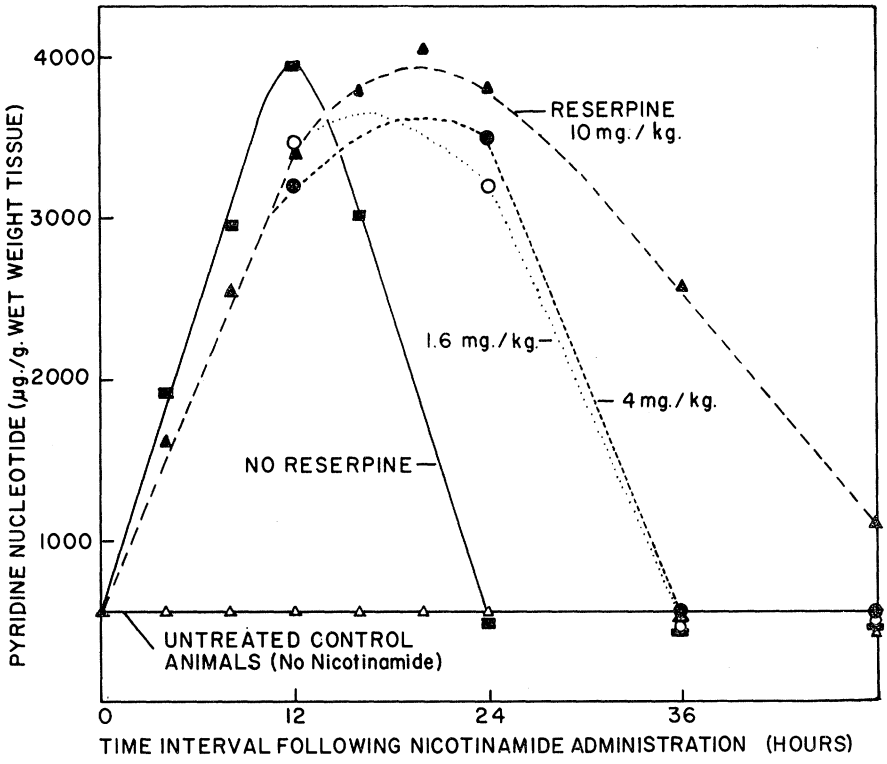

Fig. 1. Effect of reserpine upon the level of diphosphopyridine nucleotide in mouse liver. The experiment was conducted as described in the text. Open triangles, data obtained from untreated control animals and from animals that had received only reserpine; solid squares, data for animals that had received nicotinamide and nicotinamide-and-vehicle (*7*). Data from mice that had received injections of reserpine 4 hours prior to the injection of nicotinamide are indicated by the following symbols: solid triangle (10 mg/kg), solid circle (4 mg/kg), open circle (1.6 mg/kg). Each experimental point represents the average value obtained from three animals.

It is of interest to consider some possible implications of these results. First, the relationship between reserpine or chlorpromazine and the tissue DPN level following administration of nicotinamide suggests that the pyridine nucleotide may be implicated in the mechanism of action of these "tranquilizing" drugs. If a mechanism for the action of "tranquilizing" agents does involve DPN, then it may be possible to demonstrate synergism between the "tranquilizing" agent and nicotinamide. Preliminary behavioral evidence in man obtained by C. Kornetsky suggests such a relationship (*8*).

Second, in view of the recent demonstration that reserpine is an antileukemic agent (*9*) and that an analog of nicotinamide, 3-acetylpyridine, will form the corresponding analog of DPN in neoplastic tissue (*10*), it would be of interest to know whether or not nicotinamide enhances reserpine antitumor activity or whether reserpine can facilitate the formation of the DPN analog in tumor tissue. Such studies would be of practical importance in studying the mechanism of action of reserpine as an antileukemic agent.

Third, little is known of the enzymatic pathways of the synthesis of DPN. The use of agents such as reserpine and promazine may be of considerable value in elucidating the individual enzymatic steps. Since the extent of DPN synthesis was not appreciably affected by administration of reserpine, the action of the drug may be directed through the DPN-splitting enzymes. It has been found, however, that reserpine does not inhibit the breakdown of DPN by mouse-liver homogenates. This suggests that the tranquilizer does not act directly on the DPN cleavage enzymes (*11*).

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3. We wish to thank the Wyeth Institute for Medical Research for the gifts of the phenothiazine derivatives and the meprobamate (Equanil).

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Purification and Properties of an Interstitial Cell-Stimulating Hormone from Sheep Pituitaries

Two groups of investigators have previously reported procedures for the isolation of interstitial cell-stimulating hormone (ICSH) from both sheep (*1*) and swine pituitary glands (*2*). Recently, reexamination of these procedures has led us to the development of a new method utilizing some of the newer techniques for purification of proteins that have been developed since the earlier purifications were described. In the course of this work, it was noted that more than one protein component in the extract from sheep pituitaries possesses ICSH activity. Moreover, one of these active components, the isolation of which is described herein, has properties different from those reported for either of the earlier preparations (*1, 2*).

Acetone-desiccated sheep pituitaries (1 kg) were extracted (*3*) with a 0.5 percent NaCl solution at *p*H 4.6. The extract was adjusted to *p*H 7.15, and ethanol was added at −15°C until the concentration of ethanol reached 40 percent by volume. The resulting precipitate was extracted with water at 0°C, and the insoluble portion was removed by centrifugation. Solid NaCl and a 0.2*M* sulfosalicylate buffer (*p*H about 3.6) were added to the supernatant fluid to give a final concentration of 0.15*M* with respect to sodium chloride and 0.02*M* with respect to sulfosalicylate. The *p*H was then adjusted to 3.60 ± 0.03 by addition of 1*M* HCl. The precipitate that formed was of low activity and consequently was discarded; the supernatant fluid was adjusted to *p*H 6.9 and fractionated with $(NH_4)_2SO_4$. The fraction that precipitated at concentrations of $(NH_4)_2SO_4$ between 1.3*M* and 2.1*M* was collected, dialyzed, and lyophilized. This fraction (designated as fraction B) was next subjected to chromatography on a polycarboxylic acid resin, Amberlite IRC-50 (XE-97), which had been equilibrated with 0.2*M* potassium phosphate buffer of *p*H 5.88. The active fraction (fraction C) which was eluted from the column with 0.2*M* phosphate buffer of *p*H 6.9 was recovered and further purified by chromatography on an IRC-50 resin column under the conditions shown in Fig. 1. It was noted that materials in tubes 126 to 143 and in tubes 144 to 159 were equally active; these were designated as fractions D-2 and D-3, respectively.

Further purification of fraction D-3 was achieved by zone electrophoresis on starch. The contents of segments No. 19 to 29 (fraction E-3, Fig. 2) were combined, and the protein was recovered and subjected again to chromatography on IRC-50 resin under the same conditions as those shown in Fig. 1. The active fraction (obtained in a yield of 65 mg/kg of pituitary glands) obtained in this manner behaves as a homogeneous protein according to chromatography on IRC-50 resin and carboxymethyl cellulose columns, in zone electrophoresis on starch, in boundary electrophoresis by the conventional method, and in the ultracentrifuge. It represents one of the ICSH-active components in the glandular extract and is hereafter designated as β-ICSH.

The interstitial-cell stimulating activity of β-ICSH was estimated by its ability to increase the weight of the ventral prostate in hypophysectomized rats (*4*). It was found that a total dose of 0.002 mg causes an increment of ventral prostate weight of from 8.5 to 18.6 mg when it is injected intraperitoneally over a period of 4 days into 23-day-old hypophysectomized rats (Long-Evans strain, 2 days postoperatively). It is essentially free from lactogenic, somatotropic follicle-stimulating, thyrotropic, and adrenocorticotropic activities.

The highly purified β-ICSH was subjected to biophysical investigation. The electrophoretic mobility was determined at 1°C in buffers of 0.1 ionic strength over a wide range of *p*H by means of the free boundary technique. At *p*H 4.2 in acetate buffer, the mobility was found to be $+1.84 \times 10^{-5} cm^2 sec^{-1} v^{-1}$; the isoelectric point was at *p*H 7.3. The sedimentation constant and diffusion coefficient were measured in a buffer consisting of 0.010*M* K_2HPO_4, 0.010*M* KH_2PO_4, and 0.200*M* NaCl. The respective values, corrected to zero protein concentration and 20°C, were found to be $s^0_{20} = 2.47$ *S* and $D^0_{20} = 7.54 \times 10^{-7} cm^2 sec^{-1}$. From these constants, together with an assumed value for partial specific volume (0.73 ml/g) and a solvent density of 1.010, the molecular weight of β-ICSH was computed to be 30,000. Chemical characterization of β-ICSH is in progress (*5*).

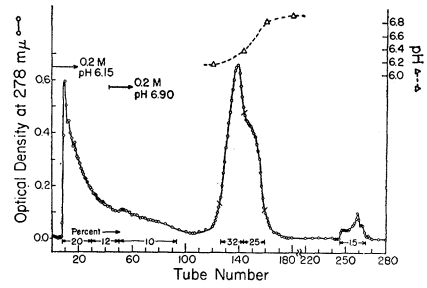

Fig. 1. Chromatography of fraction C on IRC-50 resin. One and two-tenths grams of the fraction was applied to the column containing 33 mg of resin equilibrated with 0.2*M* potassium phosphate at *p*H 6.13. Elution with buffer of *p*H 6.9 was started at tube 43. The final peak, tubes 244 to 270, was eluted with 0.2*N* NaOH. Fraction D-2 was recovered from tubes 126 to 143, and fraction D-3 from tubes 144 to 159. Percentage of recovery of protein nitrogen is given above the corresponding tube numbers. The *p*H during elution of the peak is represented by a dashed line.

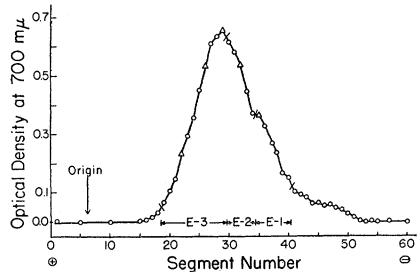

Fig. 2. Zone electrophoresis of 150 mg of fraction D-3 in acetate buffer of *p*H 4.2 and 0.10 ionic strength for 72 hours at 3.0 v cm^{-1}.

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5. This work was supported in part by grants from the National Institutes of Health, U.S. Public Health Service (No. RG 4097), and from the Albert and Mary Lasker Foundation.

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