much more effective than the methylpyridinium compound. It is to be noted that bovine serum albumin at a comparable concentration failed to bind any of these compounds, with the exception of dodecylpyridinium. (ii) The binding of curare, although salt-sensitive (compare curves 1 and 3), still occurred at a relatively high ionic strength ($\mu = 0.1$). As could be predicted from these results, a decrease in the ionic strength of the medium surrounding the electroplax resulted in a marked enhancement of the effect of curare (*6*). (iii) Addition of decamethonium and prostigmine did not result in the displacement of curare from the complex in solution. In agreement with these observations, it was found that these compounds, which depolarize the electroplax in low concentrations, are without effect on electroplax blocked by curare even when they are applied in concentrations several times as high as that of the curare (*3*).

The curare-binding component has been purified to a considerable extent by further fractionation of the extract with ammonium sulfate. A protein fraction has been obtained which, at a concentration of 0.5 mg/ml ($\mu = 0.1$; *p*H, 7.5), gives a precipitate with curare (2 mg/ml). Under similar conditions, chondroitin sulfate (3 mg/ml), deoxyribonucleic acid (2 mg/ml), and proteins of other fractions (15 mg/ml) fail to precipitate with curare. The precipitate, which contains about 50 percent of the proteins in this fraction, can be further fractionated by prolonged dialysis against 0.1 μ phosphate, *p*H 7.5. Part of the precipitate (about 20 percent) goes into solution, the remainder being solubilized by dialysis at *p*H 9. It is suggested that the latter material, which has a protein ultraviolet spectrum, may form highly specific complexes with curare and related compounds and thus has receptor-like properties. In line with the proposals of Nachmansohn and Wilson (*7*), it will be of considerable interest to determine whether those neurotropic agents considered to be "receptor activators" can induce configurational changes in this protein and whether the so-called "receptor inhibitors" are ineffective in this respect (*8*).

SEYMOUR EHRENPREIS
Department of Neurology,
College of Physicians and Surgeons,
Columbia University, New York

References and Notes

1. M. Altamirano, W. L. Schleyer, C. W. Coates, D. Nachmansohn, *Biochim. et Biophys. Acta* 16, 268 (1955); D. Nachmansohn, *Harvey Lecture Ser.* 49 (1953–54), 57 (1955).
2. C. Chagas, E. Penna-Franca, A. Hasson, C. Crocker, K. Nishie, E. J. Garcia, *Anais acad. brasil. cienc.* 29, 53 (1957); C. Chagas, E. Penna-Franca, K. Nishie, E. J. Garcia, *Arch. Biochem. Biophys.* 75, 251 (1958).
3. E. Schoffeniels and D. Nachmansohn, *Biochim. et Biophys. Acta* 26, 1 (1957).
4. This work was supported in part by the division of Research Grants and Fellowships of the National Institutes of Health (grant No. B-400) and by the National Science Foundation (grant No. G-4331).
5. I. M. Klotz, F. M. Walker, R. B. Pivan, *J. Am. Chem. Soc.* 68, 1486 (1946).
6. M. Guinnebault, unpublished observation.
7. D. Nachmansohn and I. B. Wilson, *Currents in Biochemical Research* (Interscience, New York, 1956), pp. 628–652; I. B. Wilson and D. Nachmansohn, in H. T. Clarke, Ed., *Ion Transport Across Membranes* (Academic Press, New York, 1954), pp. 35–64.
8. I wish to express my gratitude to Dr. D. Nachmansohn for his continuous encouragement and interest in this work.

22 January 1959

Enzymatic Activity of a Genetically Altered Tryptophan Synthetase in Neurospora crassa

Abstract. Partially purified preparations of certain tryptophan-requiring mutants of *Neurospora crassa,* which contain a protein (CRM) antigenically related to wild-type tryptophan synthetase, possess indole glycerol phosphate-synthesizing activity. This activity can be inhibited by anti-CRM sera. It is suggested that CRM in such mutants represents a damaged tryptophan synthetase lacking the capacity to react with L-serine.

A number of microbial mutants unable to form L-tryptophan from indole and L-serine have been described (*1–3*). Crude extracts of such tryptophan-requiring (td) mutants of *Neurospora crassa* and *Escherichia coli* contain no tryptophan synthetase activity, but in most instances the presence of large quantities of a protein (CRM) antigenically related to tryptophan synthetase can be demonstrated (*2; 4–6*). Studies with *E. coli* (*2, 6*) have shown that CRM-containing preparations from certain tryptophan-requiring mutants will catalyze the conversion of indole glycerol phosphate to indole plus triose phosphate. These results indicate that CRM in *E. coli* appears to be functionally as well as structurally related to tryptophan synthetase. Experiments to date, in which both the *Neurospora* and the *E. coli* systems were employed (*5-9*), seem to support the view that the CRM proteins may represent genetically altered tryptophan synthetase molecules.

The present report (*10*), based on antienzyme studies with the *Neurospora* system, demonstrates that the reaction:

Indole + triose phosphate → indole glycerol phosphate

which is catalyzed by certain CRM-containing preparations, can be specifically inhibited by anti-CRM serum. This antiserum also inhibits the wild-type tryptophan synthetase activity (*5*).

Strain td_2 (*1*), a tryptophan-requiring mutant that accumulates indole and indole glycerol in culture filtrates (*8*), was used for most of these experiments, although preparations from several other strains appear, on preliminary screening, to give similar results. Partially purified td_2-CRM preparations, known to catalyze the reaction (*11*)

Indole glycerol phosphate → indole + triose phosphate

are able to convert indole and triose phosphate to indole glycerol phosphate. Maximum activity occurs only in the presence of fructose diphosphate, aldolase, and pyridoxal phosphate (*12*). The methods for determining indole disappearance and indole glycerol phosphate formation have been described (*2*). Although some indole glycerol phosphate is synthesized, it is apparent that considerably more indole disappears from the reaction mixture than can be accounted for by the indole glycerol phosphate formed. In addition, the rates of

Table 1. Effect of anti-CRM serum on the enzymatic activity of CRM. A constant number of td_2-CRM (5) units was added to each of several tubes containing increasing amounts of anti-td_2-CRM serum. The tubes were stored in an ice bath for 5 minutes, followed by the addition of the substrate mixture: (7.5 μmole of indole, 10 μmole of fructose diphosphate, 40 μg of pyridoxal phosphate, $2 \times 10^{-3} M$ glutathione, 0.2 ml of crude *Neurospora* aldolase, and $0.1 M$ phosphate buffer, *p*H 7.8). The final volume per tube was 4.4 ml. Samples were incubated at 37°C for 1 hour. The reaction was stopped with 0.2 ml of 5 percent sodium hydroxide. Indole was extracted with 15 ml of toluene, and aliquots were assayed by the *p*-dimethyl aminobenzaldehyde method (*2*).

Antibody	Anti-CRM (unit)	CRM (unit)	Indole disappearing in reaction mixture (μmole)
Anti-td_2-CRM	45	77.7	0.40
Anti-td_2-CRM	60	77.7	0.02
Anti-td_2-CRM	120	77.7	0.0
td_1-absorbed anti-td_2-CRM	19	77.7	0.55
td_1-absorbed anti-td_2-CRM	38	77.7	0.45
td_1-absorbed anti-td_2-CRM	76	77.7	0.0
Anti-td_1	Equivalent volume	77.7	0.75
Nonimmune serum	Equivalent volume	77.7	0.75

indole uptake and indole glycerol phosphate formation differ significantly.

Results similar to this have been observed in experiments on indole uptake with mycelial pads of strain td_2 (*13*), suggesting that several products may be formed from indole during the course of the reaction. It should be emphasized however, that close agreement is obtained between indole uptake and the number of units of CRM in the extract, whether different aliquots of the same preparation or different preparations are compared. Furthermore, extracts of mutant strain td_1, a mutant which forms neither tryptophan synthetase nor CRM (*1, 4, 5, 14*), exhibit no "indole uptake" activity.

Rabbit anti-td_2 CRM sera (*5*) were tested for their ability to inhibit the indole reaction catalyzed by td_2-CRM-containing preparations. It was found that unabsorbed anti-td_2 CRM serum, and anti-td_2 CRM serum absorbed with extracts of strain td_1, a mutant which lacks both tryptophan synthetase and CRM (*5*) completely neutralize enzyme activity. As is shown in Table 1, anti-td_1 serum (*5*) has no inhibitory effect.

These results seem to support the proposal that the CRM protein in *Neurospora* mutant td_2 is the protein responsible for catalyzing one or more reactions involving indole. It would appear most likely that td_2-CRM represents one of a variety of possible types of genetically altered tryptophan synthetase molecules. In this case an alteration in protein structure seems to have eliminated the reactivity of the protein with L-serine, albeit an indole-combining site is still retained. It is well known that specific suppressor mutations are capable of partially restoring tryptophan synthetase activity in certain CRM-forming td mutants of *Neurospora* (*1*). Perhaps one mechanism of action of such suppressor genes involves the conversion of a CRM protein to active tryptophan synthetase by reactivating an inactive catalytic site on the CRM protein (*7, 15*).

SIGMUND R. SUSKIND
ELKE JORDAN
McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland

References and Notes

1. C. Yanofsky and D. M. Bonner, *Genetics* 40, 761 (1955).
2. P. Lerner and C. Yanofsky, *J. Bacteriol.* 74, 494 (1957).
3. M. Demerec and Z. Hartman, *Carnegie Inst. Wash. Publ. No. 612* (1956), p. 5.
→ S. R. Suskind, C. Yanofsky, D. M. Bonner, *Proc. Natl. Acad. Sci. U.S.* 41, 577 (1955).
5. S. R. Suskind, *J. Bacteriol.* 74, 308 (1957).
→ C. Yanofsky and J. Stadler, *Proc. Natl. Acad. Sci. U.S.* 44, 245 (1958).
7. S. R. Suskind and L. I. Kurek, *Science* 126, 1068 (1957).
8. J. A. DeMoss, C. Wust, A. Lacy, D. M. Bonner, personal communication.
9. C. Yanofsky and M. Rachmeler, *Biochem. et Biophys. Acta* 28, 640 (1958).
10. This article is contribution No. 257 of the McCollum-Pratt Institute. The investigation was supported by a research grant [RC-3080 (C) M and G] from the National Institutes of Health, U.S. Public Health Service.
11. J. A. DeMoss and D. M. Bonner, *Bacteriol. Proc.* (*Soc. Am. Bacteriologists*) 58, 112 (1958).
12. Either crystalline aldolase, kindly supplied by Dr. E. Racker, or crude *N. crassa* aldolase preparations were used to generate triosephosphate from fructose diphosphate. Approximately 40 percent of maximum CRM enzymatic activity was obtained without added pyridoxal phosphate.
13. S. R. Suskind, C. Yanofsky, D. M. Bonner, unpublished results.
→ C. Yanofsky, *Proc. Natl. Acad. Sci. U.S.* 38, 215 (1952).
→ S. R. Suskind and L. I. Kurek, *ibid.* 45, 193 (1959).

11 December 1958

Fat Changes during Adolescence

Abstract. Lower thoracic fat, as measured on serial chest plates of 259 children in Ohio, increased in girls, between ages 6.5 and 14.5 years and, in boys, between ages 6.5 and 11.5 years. No evidence for a marked loss of "baby" fat in adolescence or for "waves" of fattening around the time of puberty was found.

The literature on human growth refers to a number of changes in body fat during adolescence, including a loss in "baby" fat that is popularly believed to occur and the existence of several waves of fattening that supposedly occur either before or after the spurt in stature (*1*). However, currently available data are insufficient to confirm these generalizations, and the still-limited findings are contradictory.

In the present study, outer fat was investigated on the lower thorax (*2*) at the level of the tenth rib, in 259 regular participants in the Fels longitudinal program, by means of serial posteroanterior chest radiographs. Sex-specific samples ranged from 16 to 93, the age range was 6.5 to 17.5 years, and the average sample for each age group was 124. In the treatment of the data, median values were used because of the marked skewness of all 24 distributions.

As is shown in Fig. 1, there was a parallel increase in fat on the lower thorax in both sexes from the 6th through the 11th year, the median values for the girls being about 1 mm (or 40 percent) above those for the boys. Thereafter, fat on the lower thorax continued to increase in the girls, reaching a thickness of 8 to 9 mm by the 14th year, while in the boys it stabilized at about 4.5 mm between the 11th and the 17th years. No loss of "baby" fat was evident for the girls in this cross-sectional analysis, and the tiny (0.2-mm) decrease in the median values for the boys between 11 and 13 years was not statistically significant.

Further analysis of the data, on a longitudinal basis and on the basis of physiological events rather than chronological age, yielded similar results. For the girls, rearrangement of the fat measurements relative to the individual age at menarche or to the year of the maximum spurt in stature confirmed the observation that there is a fairly uniform increase in fat from childhood on. In the boys, the maximum accumulation of fat on the lower thorax generally preceded the year of the spurt in stature, and there was a suggestion that there is a small decrease about 2 years after this event.

The lack of data that would indicate a period of fat loss, or of "waves" of fattening in girls, was in agreement with earlier findings on the same population sample (*3*) and with data on fat in the calves of girls in Boston (*4*). The trends in fat on the lower thorax of boys in Ohio, while in general agreement with trends in fat at other sites in the same group (*3*), were quite different from trends in fat in the calves of Boston boys (*4*) and different from trends found by pinch-caliper measurements of boys in California (*1*). While increase in fat in the boys was noted in all three studies, there was no agreement about when it began, when it terminated, when there was a fat loss, or when there was a steady-state leveling off.

These discrepancies may be due to differences in the sites selected, since fat may increase on one part of the body and decrease simultaneously on another part during growth (*5*). Alternatively, the divergent trends apparently exhibited by California, Ohio, and Massachu-

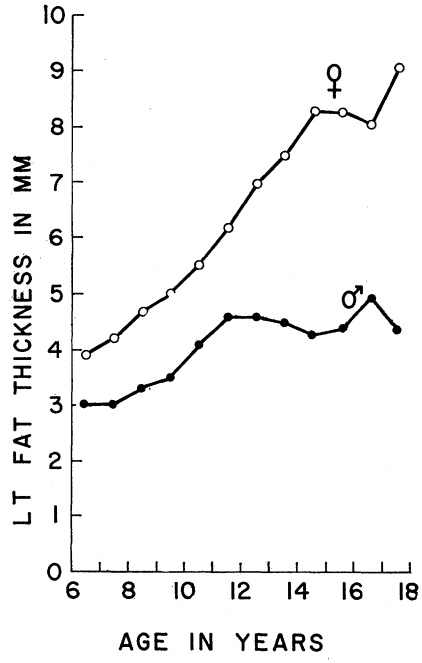

Fig. 1. Continuous increase in lower thoracic fat in girls and parallel increase in boys, terminating at 11.5 years. By 14.5 years the adult female/male fat ratio of 180 percent (*2*) has been attained.