16), digit span (*2, 3, 6, 17*), verbal reasoning (*2, 3*), size constancy (*2, 3*), and depth perception (*2, 3, 12*). Finally, hallucinatory phenomena were rare. This is in agreement with the more recent deprivation experiments (*3, 13, 18, 19*) which indicate that these phenomena are not as common as was originally believed (*1, 6*).

It is possible that our effects and their resemblance to the "deprivation" phenomena would have been even greater if a more severe condition of immobilization had been employed. The fact that all of the volunteers endured the week quite easily and that there were no failures indicates that our procedure was not particularly stressful. Furthermore, it would appear to be less stressful than prolonged isolation where approximately a third of the volunteers fail to endure periods longer than 4 days (*13, 20*).

These behavioral and physiological changes are probably mediated by the same neural mechanisms that are believed to be involved in producing the classical perceptual deprivation phenomena—that is, a disturbance of the activity of the ascending reticular activating system as a result of a decrease in the level and variability of sensory input (*6, 21*). In the present experiment, however, the decreased variability of input is from the tactile-kinesthetic (and vestibular) rather than visual and auditory senses. Interference with these sense modalities alone may be sufficient to produce certain behavioral changes, especially in the light of several reports pointing to the "powerful excitatory influence of somatic sensory excitation" upon the reticular activating system (*22*). In addition to this nonspecific reticular system, it appears that the specific sensory systems may also be involved in these behavioral effects (*23*).

These results have important implications for the numerous sensory and perceptual deprivation experiments in which the subjects are instructed to lie quietly, often for long periods of time (*1*). They raise the possibility that the behavioral deficits reported in these studies may be as much a function of restricted motility as reduced visual and auditory input, if not more. Our findings are also relevant for the treatment of hospitalized patients, particularly those whose condition is such that it requires prolonged immobilization in a cast or in an iron lung. Adverse psychological effects may occur if appropriate measures to stimulate bodily activity are not taken. The findings, incidentally, are also applicable to the "man-in-space" program. Finally, the results have some relevance for the centuries old practices of swaddling and cradling of infants. Although these practices have now virtually dispeared, they still exist among some peasants of Central Europe and Italy, as well as in Lapland and in certain North American Indian tribes (*24*). In the light of our data it is possible that these practices may produce some degree of intellectual retardation. This view is supported by Hill and Robinson (*25*), who reported a case of retarded mental development which they believed was brought about "almost entirely by the drastic restrictions made to his movements." This was a 6-year-old boy who, because of a skin condition, had severe restrictions placed on arm and leg movements. Although this is a single case, the results, nevertheless, are suggestive. These swaddling and cradling practices may also retard perceptual development, particularly in view of Riesen's (*10*) demonstration of perceptual deficits in kittens and primates who were merely restrained in holders (*26*).

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References and Notes

1. D. W. Fiske, in *Functions of Varied Experience*, D. W. Fiske and S. R. Maddi, Eds. (Dorsey, Homewood, Ill., 1961), p. 106.
2. J. P. Zubeck *et al.*, *Can. J. Psychol.* **14**, 233 (1960); **15**, 83 (1961).
3. J. P. Zubek *et al.*, *Perceptual and Motor Skills* **15**, 171 (1962).
4. J. P. Zubek, G. Welch, M. G. Saunders, *Science* **139**, 490 (1963).
5. J. P. Zubek and G. Welch, *ibid.*, p. 1209.
6. W. Heron, in *Sensory Deprivation*, P. Solomon *et al.*, Eds. (Harvard Univ. Press, Cambridge, Mass., 1961), p. 6.
7. D. Wexler *et al.*, *A.M.A. Arch Neurol. Psychiat.* **79**, 225 (1958).
8. G. L. Engel *et al.*, *Arch. Neurol. Psychiat.* **51**, 134 (1944).
9. A. Goldman, *Am. J. Psychol.* **66**, 613 (1953).
10. A. H. Riesen, *J. Nervous Mental Disease* **132**, 21 (1961).
11. R. Held, *ibid.*, p. 26.
12. J. Vernon *et al.*, in *Sensory Deprivation*, P. Solomon *et al.*, Eds. (Harvard Univ. Press, Cambridge, Mass., 1961), p. 41.
13. T. I. Myers *et al.*, "Progress report on studies of sensory deprivation," *Publ. U.S. Army Leadership Human Research Unit, Monterey, Calif.* (1961).
14. B. K. Doane *et al.*, *Can. J. Psychol.* **13**, 210 (1959).
15. D. W. Ormiston, "A methodological study of confinement," *Tech. Rept. 61-258, Wright-Patterson Air Force Base, Ohio* (1961).
16. J. Vernon and J. Hoffman, *Science* **123**, 1074 (1956).
17. L. Goldberger and R. R. Holt, *J. Nervous Mental Disease* **127**, 99 (1958).
18. G. E. Ruff and E. Z. Levy, *Am. J. Psychiat.* **115**, 793 (1959).
19. J. Vernon *et al.*, *Science* **133**, 1808 (1961).
20. J. Hull and J. P. Zubek, *Perceptual and Motor Skills* **14**, 231 (1962).
21. D. B. Lindsley, in *Sensory Deprivation*, P. Solomon *et al.*, Eds. (Harvard Univ. Press, Cambridge, Mass., 1961), p. 174.
22. J. D. French, in *Handbook of Physiology*, section 1, *Neurophysiology*, vol. 2, J. Field, Ed. (Williams & Wilkins, Baltimore, 1960), p. 1281; H. Bernhaut *et al.*, *J. Neurophysiol.* **16**, 21 (1953).
23. J. M. Sprague *et al.*, *Science* **133**, 165 (1961).
24. W. Dennis, in *Readings in Child Psychology*, W. Dennis, Ed. (Prentice-Hall, New York, 1951), p. 393.
25. J. C. Hill and B. Robinson, *Brit. J. Med. Psychol.* **1**, 268 (1929).
26. Supported by the Defence Research Board, Canada, project 9425-08. We thank Misses S. Oliver and G. Levins, and Dr. M. G. Saunders of the EEG Department, Winnipeg General Hospital, for technical assistance, and M. Aftanas, L. Bell, J. Flye, T. Hayashi, M. Menuck, M. Spigelman, and G. Winocur for experimental assistance at various stages of the project.

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Carbon Tetrachloride Poisoning in Rats: Alteration in Ribosomes of the Liver

Abstract. *Microsomes from livers of albino rats treated with carbon tetrachloride were compared with those from normal rats with respect to their ability to incorporate amino acid. Acute carbon tetrachloride poisoning results in depressed capacity of microsomes to incorporate amino acid. From ultracentrifugal data, there is an apparent dissociation of 79S ribosomes into 54S components.*

Carbon tetrachloride (CCl_4) causes hepatic necrosis, and a single sufficient exposure produces acute liver injury in many animal species (*1*). Injury occurs with such regularity and with such similarity of pattern that one can only conclude that some fundamental process is deranged by this toxic agent. How it acts has been the subject of considerable conjecture and experiment (*2–5*). The first subcellular elements suggested as the seat of the injury were the mitochondria (*2, 3*). Examination of the data revealed that the functional changes in the mitochondria occur at a time when the liver already shows significant damage when examined with the light microscope. With the electron microscope a much earlier and more subtle injury can be detected in the coarse endoplasmic reticulum (*3, 5, 6*). Coincident with this change there appears a depression in protein synthesis, the metabolic activity now known to be associated with the coarse endoplasmic reticulum (*5, 7, 8*). This report deals with experiments which extend the evidence for the site of action of CCl_4 in

damaging the liver; our data indicate that a change regularly occurs in a significant proportion of liver ribosomes.

The experiments were divided into two categories: (i) examination of the in vitro capacity of two reconstituted cell-free systems to incorporate labeled amino acids; (ii) examination by means of the ultracentrifuge of the distribution of particle size in the ribosome fraction.

Fasted male Sprague-Dawley rats weighing between 200 and 250 g were used as subjects. They were given 0.5 ml of CCl_4 (per 100 g of body weight) dissolved in an equal volume of mineral oil by stomach tube. The control animals were given an equal volume of pure mineral oil. All animals were placed in individual cages and permitted water as desired, but no food. Three hours after the administration of the materials the animals were killed by a sharp blow to the head. The livers were perfused *in situ* by way of the aorta with chilled 0.85 percent saline. They were then quickly removed and placed in an ice-cold medium consisting of 0.35*M* sucrose, 0.025*M* KCl, 0.005*M* magnesium chloride, buffered with 0.05*M* tris at *p*H 7.6. The livers were homogenized with four volumes of this fluid for 1 minute. The resulting homogenate was centrifuged at 12,000*g* for 10 minutes in a Servall SS1 centrifuge. In one set of experiments, this supernatant liquid was used without further modification. In a second set of experiments, the supernatant material was centrifuged for 1 hour at an average relative centrifugal force of 105,000*g* in a No. 40 Spinco rotor in a Spinco model L preparative ultracentrifuge. The liquid from the 105,000*g* centrifugation was saved as a source of *p*H 5 enzyme and soluble RNA (sRNA), and the pellets of microsomes were resuspended in a small amount of the medium used for initial homogenization.

In the first set of experiments, 2 ml of the microsome-rich 12,000*g* supernatant fluid were incubated with a mixture containing radioactive valine. When diluted with the microsome-rich suspension, the final mixture contained, per milliliter: 1 μmole of ATP, 0.15 μmole of GTP, 0.01 mg of PEP kinase, 10 μmole of PEP, 6 μmole of $MgCl_2$, 5 μmole of KCl, 4 μmole of reduced glutathione, and 10 μmole of valine-1-C^{14} with a radioactivity of 1 mc/mmole (*9*). Incubations were carried out for 30 minutes at 37°C. The reaction was stopped by adding 8 ml of ice-cold 0.35*M* sucrose with 0.025*M* KCl, 0.05*M* $MgCl_2$ containing 0.1*M* valine buffered with 0.05*M* tris at *p*H 6.5, and chilling the mixture to 0°C. The resulting suspension was centrifuged at 105,000*g* (average) for 60 minutes; the liquid was carefully drained from the pellet. The pellet was then suspended in 7 ml of 5 percent TCA by trituration. The proteins were isolated by the technique of Siekevitz (*10*).

In the second series of in vitro incubations the reaction mixture consisted of 0.5 ml of the microsomal suspension, 1.5 ml of the supernatant from the 105,000*g* centrifugation, and 0.2 ml of the same ATP-generating media. The amino acid in this case was replaced by a concentration of 0.03 μmole of tritiated valine per milliliter with an activity of 400 μc/μmole. The reactions were stopped, and the proteins were then isolated as in the previous method.

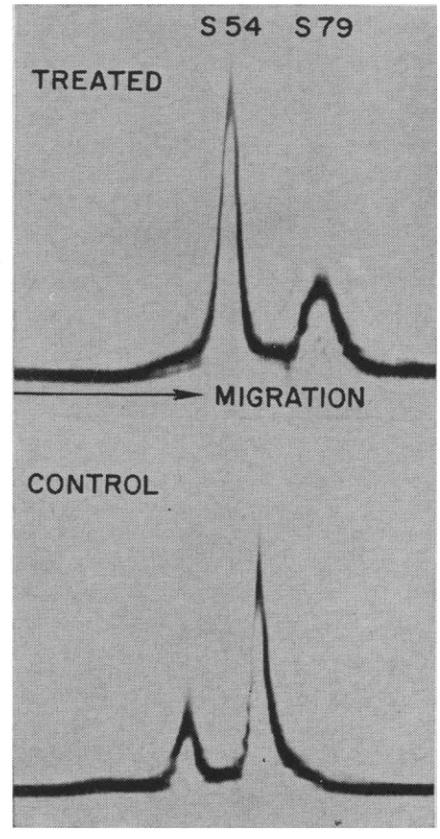

Fig. 1. Ultracentrifuge patterns of ribosomes isolated from CCl_4-treated (top) and control animals (bottom). The ribosomes were dialyzed against distilled water for 18 hours at 0°C. The pictures were taken at 16 min with a rotor speed of 37,020 rev/min. The faster peak has a corrected sedimentation constant of 79*S*, the slower peak of 54*S*.

The dried protein was weighed into low potassium-40 glass vials; it was dissolved in 1 ml of hydroxide of Hyamine (*11*), and the resulting solution was diluted with a toluene scintillating fluid containing 4 percent PPO and 0.5 percent dimethyl POPOP. The mixtures were counted in a Packard 314 EX liquid scintillation spectrophotometer. Correction for quenching and conversion of decompositions per minute was accomplished by the addition of an internal standard.

Ribosomes were isolated for ultracentrifugal and chemical analysis by the procedure of Takanami (*12*). The prepared ribosomes were redissolved in 2 ml of distilled ion-free water and dialyzed against 5 liters of distilled water, 0.001*M* $MgCl_2$ or 0.06*M* KCl, 0.005*M* $MgCl_2$, 0.05*M* tris buffer (*p*H 7.8) at 0° to 4°C for 16 hours. The resulting opalescent solutions were centrifuged at 13,000*g* in a Servall SS1 angle centrifuge for 20 minutes. The faintly opalescent supernatant solution was subjected to ultracentrifugal analysis in a Spinco model E analytical ultracentrifuge. The rotor was maintained at 20° to 24°C and the speed of centrifugation was 37,020 rev/min.

Pictures were taken at 4-minute intervals, and sedimentation coefficients were determined; the constants were corrected for concentration effects by use of data of Hamilton and Petermann, and of Keller, Cohen, and Wade (*13*). No correction was made for the Johnston-Ogston effect. The RNA was determined by the orcinol method, and protein was determined by the Folin-Ciocalteau method on aliquots of the solution analyzed centrifugally (*14*).

In the first set of experiments there was a depressed incorporation of radioactive amino acid into proteins isolated from the microsome-rich fraction of the CCl_4-treated animals as compared to that of the controls. Microsomes from four control animals incorporated amino acids to the extent, on the average, of 113 (± 39) decompositions per minute per milligram of material isolated. Microsomes from four treated animals incorporated amino acids to the extent of only 59 (± 16) decompositions per minute per milligram. The values are statistically significant ($P < 0.05$). In order to decide whether the aberration was in the sediment or in the soluble portions of the reconstituted system, the second set of experiments was performed. Here liver microsomes from control animals were

Table 1. Results of three experiments on microsomes and the supernatant from the centrifugation at 105,000*g*. Valine-2,3-H^3 with an activity of 400 mc/mmole was used as the tracer. Activity measurements were converted to decompositions per minute (dpm) per milligram of protein per milligram of protein in the ribosomal suspension by addition of an internal standard.

Source		10^{-4} dpm
Microsomes	105,000*g* Supernatant	
Control	Control	1.63 ± 0.10
Control	Treated	1.75 ± 0.21
Treated	Control	0.35 ± 0.17
Treated	Treated	0.35 ± 0.17

incubated with the liver supernatant fraction from control or experimental animals, and microsomes from experimental animals were incubated with supernatant from control or experimental animals. The results of this series of experiments (Table 1) are expressed as decompositions per minute per milligram of protein per milligram of protein in the microsomal suspension. This was done in order to account for variations in concentrations of microsomal suspensions from experiment to experiment. The data show that the depressed rate of incorporation of amino acids into microsomal material is a property of the microsomes of the CCl_4-treated animals regardless of the source of the supernatant material containing the activating enzyme, the transfer enzyme, and the sRNA. Furthermore, it is apparent that the supernatant material isolated from CCl_4-treated animals does not inhibit the capacity of the microsomes of control animals to incorporate amino acid.

The previous data, designating the ribosomes as the site of a change induced by CCl_4-intoxication, suggested examination of the physical properties of the ribosome. After intoxication of the animals, there is in the liver extracts a marked decrease in the relative concentration of material in the 79*S* peak and an increase in the slower 54*S* fraction (Fig. 1). In repeated analyses the decrease in the 79*S* peak appears equal to the increase in the 54*S* peak without significant changes in either the faster or slower sedimenting components.

The evidence now seems secure that ribosomes are the structures common to all cells where the ultimate assembly of amino acids into protein takes place. Moreover, ribosomes from organisms as varied as *Escherichia coli*, pea seedling, rat liver, and rabbit reticulocytes all seem to be composed of a series of particles, and the sizes of particles from each of these sources are similar. The larger particles appear to be formed of aggregates of the small particles and the distribution of particle populations can be reversibly altered (*15*). Finally, it has been found that the capacity of a ribosomal preparation to incorporate amino acids is dependent upon the state of aggregation of the particles. In particular, active incorporation of amino acids appears to be associated with the presence of the larger aggregates, 79*S* or greater. The effects of CCl_4, administered in vivo, on rat-liver ribosomes provides new evidence to substantiate these views.

The apparent dissociation of 79*S* particles into 54*S* particles, indicated by the reciprocal increase in the latter in the CCl_4-treated animals, seems to indicate that some special part of the cell-organelle structure is affected by the CCl_4. Experiments in which CCl_4 was applied to the homogenate in vitro did not produce a similar alteration in the sedimentation pattern. Hence the chemical defect does not appear to be a simple physical solvation of the ribosome. The ultimate chemical nature of the change produced in the ribosomes by CCl_4 remains to be discovered (*16*).

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References and Notes

1. V. A. Drill, *Pharmacol. Rev.* **3**, 1 (1951).
2. G. S. Christie and J. D. Judah, *Proc. Roy. Soc. (London) Ser. B* **142**, 241 (1954).
3. C. Oberling and C. Rouiller, *Ann. Anat. Pathol.* **1**, 401 (1956).
4. P. Neubert and D. Maibauer, *Arch. Exptl. Pathol. Pharmakol.* **235**, 291 (1959); R. O. Recknagel and B. Lombardi, *J. Biol. Chem.* **236**, 564 (1961).
5. E. A. Smuckler, O. A. Iseri, E. P. Benditt, *J. Exptl. Med.* **116**, 55 (1962).
6. M. Bassi, *Exptl. Cell Res.* **20**, 313 (1960).
7. E. A. Smuckler, O. A. Iseri, E. P. Benditt, *Biochem. Biophys. Res. Commun.* **5**, 270 (1961).
8. ———, *Federation Proc.* **21** (1962).
9. Abbreviations and source of materials: ATP, adenosine triphosphate; GTP, guanosine triphosphate; PEP, phosphoenolpyruvic acid; PEP kinase, phosphoenolpyruvic kinase; PPO, 2,5-diphenyloxazol (Packard); dimethyl POPOP, (Packard), 1, 4-bis-2-(4 methyl-5-phenyloxazolyl)-benzene; TCA, trichloroacetic acid; radioactive valine, labeled with both H^3 and C^{14}, from Volk Radiochemical Co., Skokie, Ill.
10. P. Siekevitz, *J. Biol. Chem.* **195**, 549 (1952).
11. Hydroxide of Hyamine 10-X (1*M* solution in methanol), PPO, POPOP, from Packard Instrument Co., LaGrange, Illinois.
12. M. Takanami, *Biochim. Biophys. Acta* **39**, 118 (1960).
13. M. G. Hamilton and M. L. Petermann, *J. Biol. Chem.* **234**, 1441 (1959); P. Keller, E. Cohen, R. Wade (in press).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951); W. Mejbaum, *Z. Physiol. Chem.* **258**, 117 (1939).
15. G. C. Webster, *J. Biol. Chem.* **229**, 535 (1957); H. Sachs, *ibid.* **233**, 650 (1958); A. Tissières, D. Schlessinger, F. Gros, *Proc. Natl. Acad. Sci. U.S.* **46**, 1450 (1960); P. O. P. Ts'o and J. Vinograd, *Biochim. Biophys. Acta* **49**, 113 (1961).
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All-or-None Learning and the Role of Repetition in Paired-Associate Learning

Abstract. *The learning of a list of stimulus-response items is a two-stage process involving response learning and association. It is assumed that both stages are learned in an all-or-none fashion. Subjects were trained to learn a list of paired-associate items with Rock's substitution procedure. Their performance could be predicted from the all-or-none theory with parameter estimates based upon the performance of a different group of subjects who learned the same items under normal conditions.*

In paired-associate learning the learning material is arranged in pairs consisting of a stimulus item and a response item. The stimulus-response pairs are first presented together and then the stimulus item is shown alone and the subject is asked to give the response item. Normally item pairs are repeated until the subject always gives the correct response. With the substitution procedure, however, an item pair is replaced with a new one whenever the subject fails to give the correct response.

Rock (*1*) found that this procedure did not retard learning when compared to the usual procedure of repeated presentation of all items. From this finding he concluded that learning was not gradual but an all-or-none phenomenon, since replacing "wrong" items should lead to slower learning if learning were incremental. The idea of all-or-none learning has achieved a certain popularity in the meantime, but Rock's initial evidence has been questioned by recent investigators. A process of item selection confounded the results obtained with the substitution procedure. Difficult items were not learned and were therefore replaced by new items in the substitu-