analyses, while not conclusive due to difficulty in removing the salts, suggested that the substance was a monoether of inositol and tocopherol. The material was stable in alkaline solution, but an acidity of pH 2.0 or lower split the substance and destroyed the activity on creatinuria. Direct comparisons can not be made but in rough figures, the substance was 2,500 times as effective as wheat germ oil obtained by ethylene dichloride extraction and 40,000 times as effective as wheat germ itself.

In one patient with muscular dystrophy of moderate severity, a single dose of 60 mg given with alkali reduced the creatinuria appreciably in 24 hours, and to one half the control level in 3 days. The excretion of creatine continued at this low level for a period of 8 days and then gradually rose to its previous control level. However, the effect was smaller and of shorter duration in 4 other patients with rapidly progressive symptoms. In one of these subjects a single dose of 95 mg lowered the creatine output only 14 per cent. for 3 days.

Although tocopherol or inositol given alone was without effect on creatinuria, definite effects were observed in 5 of 7 patients when these two substances were given together in equimolecular amounts for 1 or 2 days. The dosage of the mixture required to produce effects on creatinuria appeared to be proportional to the rate of progression of the dystrophic process. In general, the mixture was from 1/8 to 1/30 as effective as the condensation product. Incubation with an extract of hog stomach and duodenum increased the effect of the mixture of tocopherol and inositol on creatinuria; this effect was greater than that of equivalent amounts of tocopherol and propylene glycol similarly treated.

The observations suggest that tocopherol forms a condensation product with inositol in the gastrointestinal tract (tocopherol-inositol ether) and that the inherited defect in muscular dystrophy is a deficiency in this reaction of condensation. The degree of this deficiency appears to determine the rapidity with which muscular disability progresses. Patients in whom the disease process is mild can synthesize sufficient amounts of the condensation product when large amounts of both tocopherol and inositol are given together, but those in whom the disease is more rapidly progressive will probably require the condensation product itself.

A complete report with details of data and acknowledgments is in preparation. Investigations on the effect of prolonged administration of this product on clinical status in a large series of patients are in progress.

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MITOSIS IN REGENERATING LIVER[1]

IN one-month-old rats, the rate of mitosis is at a maximum 24 hours after partial hepatectomy.[2] The experiments described here were designed to determine whether this maximum rate could be further increased. All substances to be tested were administered intravenously 24 hours postoperatively and the remaining liver removed for assay 3 hours later. In each experiment 5 to 10 animals were given the test substance, while an equal number of controls received normal saline. Nuclei were isolated by the citric acid method and counts made with a hemocytometer.[3] For simplicity, only nuclei in metaphase and anaphase were classified as being in mitosis. Variations of 10 per cent. in the mitotic count were within the limits of error of the method and were therefore not considered significant.

Of 9 preparations of chromatin[4] from rat liver which were tested, 7 produced an increase in mitosis of 25 to 100 per cent., one showed an increase of only 12 per cent., while another gave a decrease of 13 per cent. Chromatin from beef liver gave an increase of 70 per cent., and of two preparations of rabbit liver chromatin tested, one increased the mitotic rate by 55 per cent., the other 290 per cent. Several preparations of chromatin made at room temperature all had no effect on mitosis.

When isolated chromatin was extracted with 1 M NaCl a considerable portion remained insoluble. Four preparations of this insoluble fraction were tested and none found to produce a significant change in the rate of mitosis. Of seven preparations of the soluble fraction, two gave increases of only 19 to 22 per cent., while the remainder showed increases of 60 to 140 per cent. When stored at 5 to 10° C, two of the active soluble fractions lost their stimulating effect in 2 to 4 days. One preparation of fat-free chromatin produced a 200 per cent. increase in mitotic rate.

The following substances were found to have either no effect or a negative one: Various crude fractions from the liver other than chromatin, casein digests (Stearn's Amino Acids, Amigen), l-cysteine, dl-methionine, insulin, adenosine triphosphate, adenylic acid, lecithin, biotin, lipid from chromatin.

The increase in the number of nuclei in metaphase and anaphase is not in itself sufficient evidence for an increase in the rate of mitosis, for such a result may be obtained if mitosis is arrested at either of these stages. Counts were therefore made of the relative

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of California.

[2] A. Marshak and R. Byron, Jr., unpublished.

[3] A. Marshak, *Jour. Gen. Physiol.*, 25: 275–291, 1941.

[4] A. Claude and J. S. Potter, *Jour. Exp. Med.*, 77: 345–354, 1943.

frequency of nuclei in all stages of the mitotic cycle *i.e.,* resting stage, prophase, metaphase, anaphase and telophase. The proportion of nuclei in anaphase as compared to metaphase, and of both these stages to prophases in chromatin-tested animals was found to be the same as in the controls. It was concluded, therefore, that the observed increase in the frequency of metaphases and anaphases was due to a true increase in the rate of mitosis, and that the phase of the nuclear cycle in which the stimulation occurred was some portion of the resting stage.

To determine whether material from chromatin administered intravenously can become incorporated into the nuclei of the liver, chromatin was prepared from animals which had received P^{32} as Na_2HPO_4 and this injected into test rats 24 hours after partial hepatectomy. Related substances were similarly labeled, isolated and administered to the test animals. The livers were perfused and nuclei isolated 3 hours later. Table 1 shows the accumulation of P^{32} in the liver tissue

TABLE 1

P^{32} IN REGENERATING LIVERS THREE HOURS AFTER INTRAVENOUS INJECTION OF LABELLED SUBSTANCES

Material injected	Number of rats	P^{32}/gm liver as per cent. dose	Standard error of mean
1. Inorganic phosphate	84	4.52	0.083
2. Inorganic phosphate	10	4.36	0.084
3. Rat chromatin	19	26.30	0.670
4. Lipid (rat chromatin) ..	15	32.20	0.390
5. Fat free chromatin (rat)	5	7.10	0.480
6. Adenosine triphosphate .	7	4.43	0.320
7. Rabbit chromatin	8	23.10	0.200
8. Rabbit chromatin soluble in 1 M NaCl	8	16.20	0.470

from the various substances administered. There is a large accumulation from chromatin and from phospholipid which is undoubtedly due to the particulate nature of these materials. Table 2 gives the P^{32} con-

TABLE 2

P^{32} UPTAKE BY NUCLEI

Substance injected	Per cent. dose gm nuclei	Per cent. liver P^{32} in nuclei
Inorganic phosphate	1.54	2.1
Rat chromatin	5.08	1.2
Rabbit chromatin	5.06	1.3
Rabbit chromatin soluble in 1 M NaCl	4.23	1.6
Fat free chromatin (rat) .	5.45	4.6
Lipid (rat chromatin) ...	5.15	1.0

centration in the liver nuclei as per cent. injected dose per gram nuclei, and also the nuclear P^{32} as per cent. of the total liver P^{32}. From the latter figures it is clear that P^{32} from chromatin becomes incorporated into liver nuclei most rapidly from fat-free chromatin, least rapidly from lipid, with inorganic phosphate and crude chromatin in an intermediate position. The greater concentration of P^{32} in nuclei from animals receiving chromatin can not therefore be attributed to the mere accumulation of the particulate matter in the liver. The P^{32} from the chromatin evidently does not enter the cellar nucleus as phosphate ion but as a compound that may be of considerable size and complexity. The results are in some respects analogous to those of previous experiments which showed that the nucleoprotein of the living nucleus is in a state of dynamic equilibrium in which portions of it are constantly being removed and replaced.[3]

That the mitosis stimulating property of chromatin is not confined to the liver is indicated by preliminary experiments with standardized skin wounds of the rat. When a saline extract of fat free chromatin was applied locally, granulation tissue appeared 2 to 3 days earlier than in the controls, and also filled the wound area sooner. An excess of granulation tissue was not formed and there was no evidence of irritation or inflammation.

CONCLUSIONS

(1) Chromatin contains some factor which stimulates the rate of mitosis in the liver.

(2) Radioactive phosphorus becomes incorporated into nuclei of regenerating liver more rapidly from fat free chromatin than from phospholipid or inorganic phosphate.

(3) Since phosphorus containing compounds of considerable size and complexity may enter the cell and become built into the nucleus, the results suggest a possible means whereby controlled gene mutations may be produced if the gene be considered a nucleoprotein complex. The replacement of a portion of the nucleic acid or nucleotide by another of different structure may produce a change in function in the region of the chromosome so effected. Changes in type of pneumococcus[5] may be accounted for by such a process, rather than by alteration of the cytoplasm, as has been suggested.[6]

(4) Preliminary results indicate that a derivative of chromatin stimulates the rate of formation of granulation tissue in skin wounds.

Details of these experiments will be published elsewhere.

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SURVIVAL OF THE MAMMARY TUMOR MILK AGENT OF MICE[1, 2]

PREVIOUS observations demonstrated that the milk agent or inciter of mammary tumor in mice, normally

[5] O. T. Avery, C. M. MacLeod and M. McCarthy, *Jour. Exp. Med.*, 79: 137–157, 1944.

[6] T. H. Sonneborn, *Proc. Nat. Acad. Sci.*, 29: 338–343, 1943.

[1] Preliminary report.