Biochemical Lesion in Dilantin-Induced Erythroid Aplasia

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Many drugs are capable of causing bone marrow injury. No clear biochemical explanation has been discovered. Some disturbance in cell metabolism must be involved, yet attempts to demonstrate a direct in vitro effect on bone marrow cells have generally met with failure unless the offending drugs were used in concentrations far exceeding therapeutic levels. Since among the population exposed to a given drug only a rare individual gets afflicted with bone marrow toxicity, it is highly probable that an individual susceptibility underlies the metabolic handicap. Studies directed at testing this hypothesis must thereby be performed on patients who have bone marrow aplasia from a drug or who have recovered from it. Described here are clinical and biochemical investigations on a patient with reversible erythroid aplasia induced by 5-5' diphenylhydantoin (Dilantin). The results indicate that Dilantin exerted its toxic effect in this patient by specifically inhibiting DNA synthesis in erythroid precursors, probably at the step of deoxyrihotide formation.

Case History

The clinical report of this case has been previously published and is accordingly summarized here only briefly:

This 17-year-old Negro boy was admitted to the St. Louis City Hospital on September 13, 1962, because of increasing weakness, lethargy and pallor of 4-week duration. The patient was known to have had a convulsive disorder since infancy, to be mentally...
Fig. 1.—Initial course of patient's hospitalization showing induction of red cell aplasia by Dilantin and recovery when drug was discontinued. Complete red cell aplasia was produced with 300 mg. Dilantin daily for 2 weeks. Daily doses of 30 and 60 mg. of the drug caused red cell hypoplasia without disappearance of nucleated red cells from the marrow. The administration of phenobarbital and thioridazine (Mellaril) had no effects.

defective, and to have a serious behavior problem. On March 10, 1960, he was admitted to the St. Louis State Training School and Hospital and was begun on Dilantin, 300 mg. daily, and phenobarbital, 90 mg. daily. On January 30, 1961, the daily Dilantin dose was increased to 400 mg. and the phenobarbital dose to 135 mg. On July 30, 1962, he was also begun on thioridazine (Mellaril), 75 mg. daily. Blood counts in March 1960 and June 1961 showed no anemia.

At the time of admission the patient was acutely ill and extremely pale. His blood pressure was 75/42, pulse 106 per minute, temperature 100.5 F., and respirations were 18 per minute. There were numerous hemorrhages in both retinas. His left fourth toe was 10 mm. shorter than his left fifth toe. There were no other pertinent physical findings.

Laboratory examinations revealed the following: Hemoglobin 2.2 Gm. per cent; hematocrit 7 per cent; reticulocytes 0.0 per cent; platelets 303,000/cu. mm.; white blood cell count 10,000/cu. mm., with 54 per cent segmented neutrophils, 3 per cent band forms, 30 per cent lymphocytes, 7 per cent monocytes, 5 per cent eosinophils, and 1 per cent basophils. Although cellular, the aspirated bone marrow was remarkable in the complete absence of nucleated red cells and an increase in eosinophils. Serum iron was 213 µg. per cent; total iron binding capacity was 240 µg. per cent; 24-hour urine erythropoietin assay showed a level six times above normal. All other laboratory data, including hemoglobin electrophoresis, serum vitamin B₁₂ and folic acid levels, chromosomal studies, electrolytes, blood urea nitrogen, fasting blood sugar, and electrocardiogram were normal.

The hospital course is summarized in Figure 1. On admission all drugs were discontinued and the patient was given 250 ml. of packed red cells on days 1, 2 and 4. Reticulocytes appeared in the peripheral blood on day 9 reaching a maximum of 10.2 on day 19. Bone marrow examination on day 12 revealed erythroid hyperplasia. Complete recovery ensued.

As illustrated in the figure, the readministration of 300 mg. Dilantin daily for 14 days resulted in complete disappearance of reticulocytes from the peripheral blood and of nucleated erythroid cells from patient's bone marrow. Complete recovery again followed discontinuation of the drug. The administration of Dilantin for the third time in a daily dose of 30 mg., then 60 mg. daily, again resulted in severe depression of erythropoiesis—
however, only after 30 days and without complete disappearance of red cell precursors. Complete recovery again occurred when the drug was discontinued. Finally, the administration of both phenobarbital and Mellaril had no hematologic effects.

**Materials and Methods**

Uniformly labeled glycine C\(^\text{14}\) \(10 \, \mu\text{c.}/\mu\text{mole}\), Na formate-C\(^\text{14}\) \(4 \, \mu\text{c.}/\mu\text{mole}\), thymidine-2-C\(^\text{14}\) \(5 \, \mu\text{c.}/\mu\text{mole}\) and orotic acid-C\(^\text{14}\) \(4 \, \mu\text{c.}/\mu\text{mole}\) were obtained from New England Nuclear Corporation, Boston, Mass. Deoxyuridine-2-C\(^\text{14}\) \(2 \, \mu\text{c.}/\mu\text{mole}\) was obtained from Nuclear Chicago, Chicago, Ill. Unlabeled deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine were obtained from Schwartz BioResearch, Inc., Orangeburg, N.Y. Diphenylhydantoin sodium was purchased commercially. Rabbit antihuman globulin labeled with fluorescein isothiocyanate was obtained from Microbiological Associates, Bethesda, Maryland.

All glassware was coated with silicone. Bone marrow samples obtained from the patient and from normal donors were prepared for studies by gentle aspiration into, and ejection from, a syringe. Marrow cells were suspended and incubated in donor's own plasma anticoagulated with 0.02 mg./ml. heparin. The nucleated cell count varied slightly (20-25,000/cu. mm.) from one experiment to another but was kept constant for each one experiment.

Cell suspensions were distributed in 1.8 ml. volumes into 25 ml. flasks. Dilantin was added in a volume of 0.1 ml. to give a final concentration of 0.2 \(\mu\text{g./ml.}\). Control flasks received 0.1 ml. saline. Following incubation for 15 minutes at 37\(\text{C}\), the flasks had added to them radioactive precursors (C\(^\text{14}\) labeled glycine, lysine, formate, adenine, orotic acid, deoxyuridine, or thymidine) in a volume of 0.1 ml. to give a final concentration of 0.2 \(\mu\text{mole}\) per ml. The flasks were glass-stoppered and incubated at 37\(\text{C}\) in a Dubnoff metabolic shaker for 3 hours. All experiments were carried out in duplicate.

**Incorporation of Purine and Pyrimidine Precursors into Nucleic Acids.** The technics employed for the isolation of RNA, DNA and DNA bases have been previously described.\(^2\) After incubation, the suspensions were washed once with ice cold 0.85 per cent saline, and the red cells were hemolyzed in 3 per cent acetic acid. After precipitation of the nucleic acids and proteins with 10 per cent trichloroacetic acid (TCA), the lipids were extracted with cold ether and alcohol.\(^3\) The nucleic acids were extracted in 10 per cent NaCl in the cold at neutral pH, followed by extraction at 100\(\text{C}\) for 30 minutes. The sodium nucleates were precipitated with 2.5 volumes of cold 95 per cent ethanol. The precipitate was dissolved in 2 ml. of 0.1 N NaOH and incubated for 24 hours at 37\(\text{C}\) to hydrolyze the RNA. Deoxyribonucleic acid was precipitated from this hydrolysate with 2 N HCl in the cold. Hydrolysis of the residual RNA in 0.1 N NaOH at 80\(\text{C}\) for 30 minutes and precipitation of the DNA were carried out 3 times.

DNA was hydrolyzed in 12 N perchloric acid in a boiling water bath for 1 hour, and the hydrolysate was then neutralized with 6 N KOH. The DNA bases were separated by ascending chromatography on Whatman No. 1 filter paper with an isopropanol HC1 solvent,\(^5\) eluted with 0.1 N HCl, and quantitated according to the method of Bendich.\(^6\) Eluates of spots from adjacent areas served as controls.

For the determination of radioactivity, 0.4 ml. of DNA, RNA, or thymine solution was evaporated to dryness on stainless steel planchets, and was assayed in a thin-window gas-flow counter. The results were expressed as counts per minute per 100 \(\mu\text{g.}\) nucleic acid or counts per minute per \(\mu\text{mole}\) thymine at infinite thinness.

**Uptake of Glycine-C\(^\text{14}\) into Heme.** After incubation, heme was isolated and recrystallized in glacial acetic acid saturated with sodium chloride, as described by Fischer.\(^7\) The heme crystals were dissolved in 0.1 NaOH and their radioactivity was assayed as above.

**Uptake of Glycine and Lysine into Protein.** After hemolysis of the red cells in 3 per cent acetic acid, the proteins and nucleic acids were precipitated in 10 per cent TCA. The precipitate was washed with TCA. alcohol and alcohol-ether (3:1) to remove lipid material. The nucleic acids were removed by extraction in hot (80\(\text{C}\)) 5 per cent TCA twice for 20 minutes, and the protein residue was dissolved in 0.5 N NaOH and was counted as above.

**Uptake of Tritiated Precursors.** Incubations were carried out exactly as with C\(^\text{14}\) precursors. Each flask contained 0.050 \(\mu\text{c.}\) of tritiated \(\text{H}^-\text{uridine}, \text{H}^-\text{deoxyuridine or H}^-\text{thymidine.}

Radioautographs were prepared of the buffy coat with NTC-3 nuclear track emulsion

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The effect of Dilantin and patient's plasma on a normal volunteer. No effect was noted on volunteer's serum iron, iron-binding capacity or Fe\textsuperscript{59} clearance.

(Eastman Kodak Co., Rochester, N. Y.). All radioautographs were developed at the same time of day and for exactly the same developing and fixing time.

One thousand consecutive red cell precursors (early and late erythroblasts, and normoblasts) and 1000 granulocyte precursors (myeloblasts, promyelocytes, and myelocytes) were tallied in each experiment. The total number of grains was enumerated. Background counts were estimated in 1000 areas approximately equal to that occupied by red cells or by white cells and subtracted from the total. The results were expressed as per cent cells labeled, granules per labeled cell, and granules/cell (total).

EXPERIMENT AND RESULTS

The induction of pure red cell aplasia twice with Dilantin in this patient and the absence of hematologic changes from phenobarbital and thioridazine established Dilantin as the causative agent beyond doubt. The fact that it took initially 2 years of Dilantin therapy before the appearance of anemia, while a subsequent challenging course of only 4.7 Gm. reproduced the aplasia, suggested an immunologic mechanism. However, the following were considered evidence against this possibility: absence of red cell destruction, a negative Coombs' test with and without Dilantin, and the dose-effect relationship as demonstrated by the incomplete erythroid depression resulting from a daily dose of 60 mg. Dilantin.

Effect of Patient's Plasma on Normal Recipient. In order to explore further the possibility of an immune mechanism, the following experiment was done: 1500 ml. of plasma was collected from the patient during the aplastic phase. A compatible normal volunteer was begun on 300 mg. Dilantin daily followed by 500 ml. of patient's plasma intravenously over a period of 3 hours, on day 4, 5 and 6. This had no detectable effect on recipient's reticulocyte count, number of nucleated red cells in his marrow, serum iron, iron binding capacity or Fe\textsuperscript{59} clearance (Fig. 2).

Attempts to Demonstrate Antigen-Antibody Reaction by Immunofluorescence. Patient's bone marrow cell obtained after full recovery were incubated
Fig. 3.—The effect of Dilantin on the uptake of C\textsuperscript{14}-formate into DNA, DNA thymine and RNA in patient’s bone marrow and in control marrow. A 30 per cent inhibition of formate uptake into DNA and DNA thymine was observed in patient’s bone marrow at a Dilantin concentration of 20 \( \mu g./ml \). There was no inhibition of uptake into RNA. No Dilantin effect was noted on control marrow.

at 37 C. with and without Dilantin for 30 minutes in patient’s own plasma secured during the aplastic phase. Controls consisted of patient’s marrow incubated in compatible normal plasma and marrow from normal volunteer incubated in its own plasma. The cells were washed in buffered saline and suspended in saline containing 1 per cent albumin. Smears were prepared and stained for 30 minutes with fluorescin-labeled rabbit antihuman globulin. No difference in fluorescence could be demonstrated between patient’s cells and controls.

Having failed to demonstrate an immune mechanism for the Dilantin effect in this patient, attention was directed to the possibility of a direct metabolic effect exerted by this drug. Unless stated otherwise, all metabolic studies were performed on patient’s bone marrow obtained after full recovery from the aplastic phase.

Effect of Dilantin on the Uptake of C\textsuperscript{14} Formate into Bone Marrow Nucleic Acids. At a Dilantin concentration of 20 \( \mu g./ml \) there was a 25–30 per cent inhibition of formate uptake into DNA but not into RNA (Fig. 3). This experiment was repeated 3 times and the Dilantin effect was demonstrated each time. Concentrations of up to 100 \( \mu g \). Dilantin/ml had no effect on bone marrow from 10 normal volunteers; a representative experiment is shown in Figure 3.

No effect from similar concentrations of Dilantin could be demonstrated on glycine uptake into heme or glycine and lysine uptake into protein.

Specificity of Dilantin Effect for Red Cell Precursors. One of two ways (\textit{vide infra}) used to test the specificity of the Dilantin effect for erythroid precursors was to repeat the same experiment when these cells were completely
absent from the patient's bone marrow. When this was done, no Dilantin effect could be demonstrated (Fig. 4).

Effect on the Uptake of Deoxyuridine and Thymidine into DNA. The inhibition by Dilantin of the uptake of formate into DNA but not into RNA suggested a metabolic block in the conversion of deoxyuridylic acid → thymidylic acid, a step requiring methylene-tetrahydrofolate. To examine this possibility, the incorporation of C\textsuperscript{14} deoxyuridine and C\textsuperscript{14} thymidine was studied. As can be seen in Figure 5, neither the uptake of deoxyuridine nor that of thymidine was affected by Dilantin.

Effect on the Uptake of Glycine, Adenine and Orotic Acid. The absence of inhibitory effect of Dilantin on the deoxyuridine uptake into DNA or DNA thymine indicated that the site of inhibition of DNA synthesis by this drug occurred at an earlier step—perhaps the synthesis of deoxyribotides. The inhibition by Dilantin of the uptake of formate, glycine, adenine and orotic acid into DNA but not into RNA, and the absence of effect on the uptake of deoxyuridine and thymidine (Fig. 6) was strongly suggestive of such a block.

Autoradiographic studies. In order to test further the possibility of a block
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Fig. 6.—The inhibition by Dilantin of the uptake of formate, glycine, adenine, and orotic acid into DNA but not into RNA of patient’s bone marrow, and the absence of effect on the uptake of deoxyuridine and thymidine.

Fig. 7.—The effect of Dilantin on the uptake of H3-uridine, H3-deoxyuridine and H3-thymidine by erythroid and myeloid cells of patient’s bone marrow. Dilantin inhibited the uptake of uridine only by nucleated red cells. There was no inhibition in myeloid cells.

in deoxyribotid synthesis as the primary site of action of Dilantin in this patient, the incorporation of tritiated uridine, deoxyuridine, and thymidine by patient’s bone marrow cells was studied. This experiment offered another method for testing the specificity of the Dilantin effect for the erythroid precursors. As can be seen from Figure 7, 22.1 per cent of the erythroid cells were labeled with H3-uridine in the presence of 20 μg./ml. Dilantin, compared to 35.7 per cent in the control; the number of granules per labeled cell was depressed by 65 per cent in the presence of the drug. No such inhibition could be demonstrated in the myeloid elements. Dilantin had no effect on the uptake of H3-deoxyuridine or H3-thymidine.
Fig. 8.—Data on the induction and maintenance of red cell aplasia with Dilantin and the failure of large dose of vitamin B₁₂ and folinic acid to reverse the aplasia. The daily i.v. administration of deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine had a suggestive, but not a clear-cut, effect in reversing the hematologic changes. Complete recovery followed discontinuation of Dilantin therapy.

Effect of Vitamin B₁₂ and Folinic Acid on the Dilantin-Induced Hematologic Changes. Although at no time were there any megaloblastic changes observed in the patient’s bone marrow during the entire course of this study, the possibility remained that a reversal of Dilantin toxicity could be effected by the administration of large doses of vitamin B₁₂ or folinic acid. To test this, red cell aplasia was induced a fourth time in this patient by the administration of 300 mg. dilantin daily. On the twelfth day of this regimen his reticulocyte count was 0.0 per cent. A bone marrow examination on the fifteenth day revealed complete absence of erythroid precursors. Red cell aplasia was maintained thereafter with 130 mg. Dilantin daily. The course of this experiment is illustrated in Figure 8.

The rate of fall in the level of hemoglobin is entirely compatible with cessation of red cell production as the sole factor responsible (approx. 25 per cent fall in one month). Folinic acid, 18 mg. i.m. daily for 10 days, followed by vitamin B₁₂, 1000 μg. i.m. daily for 10 days, had no effect on the anemia, reticulocytopenia, or number of nucleated red cells in the patient’s bone marrow.

Effect of the Intravenous Administration of Deoxyribonucleosides. In view of the results of the in vitro studies suggesting a metabolic block in the synthesis of deoxyribotides in the presence of Dilantin, the possibility was considered that by the administration of all four deoxyribonucleosides to the patient one might bypass the site of block and thereby reverse the Dilantin effect. Accordingly, the patient was given a daily intravenous infusion of physiologic saline containing 5 grams per liter of each of the deoxynucleosides. The solutions were sterilized prior to infusion by passage through a Seitz filter. An attempt was made to give the patient a liter of the material over a period of 6 to 8 hours daily. However, depending on the degree of his cooperation, he was given the following daily amounts (in grams) of each of the compounds: 1, 2, 2, 2, 0.5,
Fig. 9.—Failure of Dilantin to induce red cell aplasia when administered simultaneously with large oral doses of riboflavin. However, patient remained refractory to Dilantin long after riboflavin was discontinued.

0.5, 1, 1, 3.3, 3.3, 5, 5, 3.3, on days 1 to 13, respectively. The results of this experiment are illustrated in Figure 8. Although no clear-cut response to this trial therapy was evident, several findings suggested that a partial response might have occurred: apparent stabilization of the hemoglobin level, appearance of few nucleated red cells in the bone marrow only 3 days after termination of therapy, and a reticulocyte response beginning on the fourth day after Dilantin was discontinued instead of the usual 7 to 9 days. It should be added that no apparent complications of any nature resulted from the infusion of the deoxyribonucleosides. The highest serum uric acid observed was 15 mg. per cent on day 12, with a return to normal level 10 days after termination of therapy. The blood urea nitrogen remained normal throughout. Here, again, complete hematologic recovery followed the discontinuation of dilantin treatment.

Effect of Riboflavin. In view of a report by Lane and Alfrey describing riboflavin responsive pure red cell aplasia, the possibility of a relation between the Dilantin-induced red cell aplasia in our patient and riboflavin metabolism was considered. It was therefore decided to start the patient simultaneously on Dilantin in increasing daily doses and on riboflavin, 120 mg. daily orally. The course of this experiment is shown in Figure 9. As can be seen from the figure, the patient was now totally refractory to Dilantin, with no hematologic changes appearing for as long as 6 months on the drug (2-month period not shown) even though riboflavin was discontinued after only 6 weeks. Three weeks after the conclusion of this experiment, bone marrow was obtained from the patient to study the uptake of formate-C\textsuperscript{14} into nucleic acids. As can be seen from Table 1, Dilantin did not inhibit the uptake for formate-C\textsuperscript{14} into DNA. Thus it was apparent that in some manner the patient had acquired resistance to the toxic effect of Dilantin. The role of riboflavin, however, remained uncertain. In order to check further the relation to riboflavin, patient was left free of all medications for 9 months, after which he was again started
Table 1.—Effect of Dilantin on the Uptake of C*-Formate into DNA of Patient's Bone Marrow. Experiment Performed after Patient Became Refractory To the Toxic Effect of the Drug

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<tr>
<th>Dilantin (µg./ml.)</th>
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* Incubations performed in triplicate.

on Dilantin alone, 300 mg. daily, and continued on this treatment for 3 months. However, now again no hematologic changes could be demonstrated.

DISCUSSION

The pathogenesis of drug-induced bone marrow aplasia is poorly understood. Although several hypotheses have been considered—including immune mechanisms, direct metabolic effect, and biochemical predisposition to the toxic effect of certain drugs—none of these hypotheses has gained significant experimental support. Pure erythroid aplasia is a rare disorder most commonly associated with thymoma. A clear-cut relation to drugs has not been found, although sulfathiazole has been seriously implicated in one case. The patient described herein, and previously reported by Brittingham et al., represents the first example of pure erythroid aplasia induced by Dilantin.

The regular reversibility of the red cell aplasia in this patient upon discontinuation of Dilantin has afforded an invaluable opportunity for study. Aplasia was induced by the drug on five separate occasions, including the initial episode, twice during his initial hospitalization (as shown in Fig. 1), once for the purpose of doing plasmapheresis (not illustrated), and once again to test the effect of vitamin B₁₂, folinic acid and deoxyribonucleosides (Fig. 8).

Perhaps most difficult to explain in this patient was the fact that it took 2 years of therapy with Dilantin to precipitate the initial episode of red cell aplasia, while subsequently the same end result could be reproduced with only 4.7 Gm. of the drug. This in itself suggested an immunologic mechanism. However, in view of the absence of any red cell destruction, the negative Coombs' test with or without Dilantin, the incomplete erythroid depression with smaller doses of the drug, the lack of effect of patient's plasma and Dilantin in a normal volunteer, and the inability to demonstrate binding of gamma globulin to patient's nucleated red cells in the presence of Dilantin, an immunologic mechanism was considered very unlikely.

The reproducible inhibition of formate-C¹⁴ uptake into DNA in patient's bone marrow by 20 µg. Dilantin/ml. and the absence of inhibition in normal marrows with drug concentration up to 100 µg./ml. suggested strongly a biochemical predisposition to Dilantin toxicity in this patient. Efforts directed to localize the site of Dilantin effect yielded some very interesting results. Since Dilantin inhibited the uptake of formate-C¹⁴ into DNA but not into RNA, two possibilities were considered: a block either in the methylation of deoxyuridylic acid to thymidylic acid, a step requiring methylene tetrahydrofolic
acid, or in the synthesis of deoxyribonucleotides; both of these metabolic steps are necessary for the complete synthesis of DNA. The absence of inhibition of uptake of deoxyuridine by Dilantin made the first possibility unlikely. The subsequent experiments, showing inhibition by Dilantin of the uptake of adenine, glycine, and orotic acid into DNA but not into RNA, and the inhibition of uptake of H\(^3\)-uridine but not of H\(^3\)-deoxyuridine, suggested strongly that the block was indeed in the synthesis of deoxyribonucleotides. Perhaps most intriguing was the specificity of this effect to the erythroid elements. That this effect was specific for red cell precursors was clearly indicated by the autoradiographic studies showing inhibition of uptake of tritiated uridine only by these cells, and the inability to demonstrate an in vitro Dilantin effect when these cells were absent from the patient's bone marrow. There is no ready explanation for this specificity, however. It is conceivable that the biochemical defect was limited to the erythroid cells; an alternative explanation would be the possible selective concentration of Dilantin by red cell precursors. It is not possible to answer these questions from the studies presented herein. An experiment wherein peripheral blood erythrocytes from the patient were incubated with C\(^{14}\) Dilantin showed no significant intracellular concentration of the drug.

Macrocytic megaloblastic anemia responsive to folic acid has been observed in patients receiving Dilantin.\(^{11}\) There was absolutely no indication in our patient that the Dilantin toxicity had any relation to vitamin B\(_{12}\) or folic acid. Serum vitamin B\(_{12}\) and folic acid were both normal; neither folic acid (0.1 \(\mu\)g./ml.) nor vitamin B\(_{12}\), 0.01 \(\mu\)g./ml., reversed the in vitro effect of Dilantin (not illustrated). Furthermore, large doses of either vitamin given to the patient did not reverse Dilantin toxicity. It should further be emphasized that in over 50 bone marrow examinations which this patient had during the entire course, at no time were there any megaloblastic changes.

Of interest was the question of hematologic response to the intravenous infusion of all four deoxyribonucleosides: deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine. Although a definite response was not evident, the apparent stabilization of the hemoglobin level, and the appearance of early erythroid cells in the marrow and reticulocytes in the peripheral blood considerably earlier than usual after discontinuation of Dilantin, suggested a partial or a beginning response. Unfortunately, this part of the experiment cannot be considered conclusive, since very frequent interruptions and, finally, a complete halt were necessary as the patient became reluctant to continue with the experiment. Thus the duration of the daily infusion varied between 2 and 8 hours. It is possible that if deoxyribosides were given as a continuous infusion (around the clock), a definite response might have occurred. Since little is known about the uptake, storage, and degree of utilization of administered deoxyribonucleosides, no conclusions can be made at this time.

The pathways of synthesis of deoxyribonucleotides in mammalian tissues are not completely understood. Evidence indicates that reduction of ribose to deoxyribose occurs at the ribotide level.\(^{12}\) Recently, an enzymatic system for the reduction of cytidine diphosphate to deoxycytidine diphosphate in rat hepatoma has been extensively studied by Moore and Reichard.\(^{13}\) It would be of great interest to examine the effect of various concentrations of Dilantin on this system. This is currently under study.
Of great interest in this patient is the development of resistance to the toxic effect of Dilantin. Whether riboflavin had anything at all to do with this resistance remains uncertain. It should be emphasized that the patient had no signs or symptoms to suggest riboflavin deficiency. The fact that he remained resistant to the hematotoxic effect of the drug long after riboflavin was discontinued (approximately a year) suggested that the appearance of resistance was coincident with, rather than a result of, riboflavin therapy.

It is quite conceivable, on the other hand, that the repeated administration of the offending agent resulted in the development of a new resistant cell line or the appearance of compensatory alternate pathways for deoxyribotide synthesis. The fact that an inhibitory effect on formate incorporation into DNA could no longer be demonstrated tends to favor this hypothesis. Pisciotta has recently offered evidence for similar compensatory mechanisms in patients susceptible to the leukopenic effect of chlorpromazine.14

Attempts to demonstrate an in vitro metabolic effect of many drugs on bone marrow cells in order to explain their in vivo toxicity have not proved successful unless such drugs were used in concentrations far exceeding therapeutic levels. For example, chloramphenicol, the most common offender in drug-induced bone marrow aplasia, had no effect on DNA, RNA or protein synthesis in normal human bone marrow unless used in concentrations of 150 μg./ml. or more.15 However, in five patients who have recovered from bone marrow aplasia thought to be due to chloramphenicol, concentrations of 25-50 μg./ml. of the drug caused significant inhibition of DNA and RNA synthesis, suggesting individual biochemical susceptibility.16 Although a difference has been noted in the in vitro effect of chlorpromazine on the uptake of tritiated thymidine in affected and normal individuals, in all instances the drug was used in relatively very high concentrations.17

We believe that the patient presented herein represents a clear-cut instance of an individual biochemical susceptibility to Dilantin since significant in vitro inhibition of DNA synthesis was consistently observed at physiologic concentrations of the drug. Furthermore, the data strongly suggest that Dilantin exerted its toxic effect in this patient by specifically inhibiting DNA synthesis at the step of deoxyribotide formation, as depicted in Figure 10. Thus, similar studies in patients who recover from drug-induced bone marrow toxicity may prove useful in uncovering the basic mechanisms involved.

**SUMMARY**

Clinical and biochemical studies are reported in a patient who developed pure red cell aplasia after 2 years therapy with Dilantin but in whom aplasia could subsequently be induced with 4-5 Gm. of the drug. Recovery occurred regularly when the drug was discontinued.

The administration of 1500 ml. of patient’s plasma obtained during the aplastic phase to a normal volunteer with Dilantin had no effect on the recipient’s reticulocyte count, number of nucleated red cells in his bone marrow, or Fe59 clearance. Attempts to demonstrate binding of gamma globulin to the patient’s nucleated red cells in the presence of Dilantin by immunofluorescence technic were unsuccessful.

Dilantin, in a concentration of 20 μg./ml. in vitro, caused significant inhibi-
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Fig. 10.—Diagram depicting the proposed site of action of Dilantin.

tion of the uptake of C¹⁴ formate, glycine, adenine, orotic acid, and uridine into DNA but not into RNA of patient's bone marrow studied when fully recovered. There was no effect on the uptake of deoxyuridine or thymidine.

The Dilantin effect was specific to the erythroid cells as shown by radioautographic studies and by the absence of inhibition when these cells were absent from the bone marrow.

Vitamin B₁₂ and folinic acid given to the patient in large doses did not reverse the hematologic effects of Dilantin. The daily intravenous administration of all four deoxyribonucleosides, deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine had a suggestive, but not clear-cut, effect.

In an effort to examine a possible relationship of the pure red cell aplasia in this patient to riboflavin metabolism, the patient was started simultaneously on Dilantin and riboflavin. Dilantin was now without effect. However, the patient remains refractory to Dilantin a year after riboflavin was discontinued.

It is concluded that Dilantin exerted its toxic effect in this patient by specifically inhibiting DNA synthesis in erythroid cells probably at the step of deoxyribotide formation.

The role of riboflavin in the development of resistance to the toxic effect of Dilantin in this patient remains uncertain.

SUMMARIO IN INTERLINGUA

Es reportate studios clinic e biochimic in un patiente qui desenvolvava pur aplasia erythrocytic post un therapia a Dilantin durante 2 annos e in qui aplasia poteva esser inducute subsecuentemente per medio de 4 a 5 g del pharmaco. Restablimento occurreva regularmente post le cessation del administrationes.

Le administration de 1500 ml de plasma obtenite ab le patiente durante le phase aplastic a un voluntario etiam tractate con Dilantin habeva nulle effecto super le numeracion reticuloцитic del recipiente, super le numero de nucleate cellulas rubie in su medulla ossee, o super su clearance de Fe⁴⁺. Effortios de demonstrar ligation de globulina gamma al nucleate erythrocytos del patiente in le presentia de Dilantin—utilisate technicals immuno-fluorescentic—remaneva sin successo.

Dilantin, in un concentration de 10 μg/ml, causava in vitro un significative inhibition del acceptation de C¹⁴ in formato, glycina, adenina, acido orotic, e uridina ad in ADN sed non ad in ARN del medulla ossee del patiente studiate post restablimento complete. Esseva notate nulle effecto super le acceptation de deoxyuridina o de thymidina.
Le efecto de Dilantin espeva specific pro cellulas erythroide, a judicar per studios radioautographic e per le absentia de inhibition quando ille celluhts esseva absente ab le medulla ossee.

Vitamin B₉ e acido folinic, administrate al patiente in grande doses, non reverteva le effectos hematologic de Dilantin. Le diurne administration intravenose de omne le quatro deoxyribonucleosidas—deoxyadenosina, deoxyguanosina, deoxyctydina, e thymidina—habeva un effecto suggestive sed non definite.

Con le objectivo de clarificar un possibile relation inter le pur aplasia erythrocytic in iste patiente e aspectos del metabolismo de riboflavina, le patiente recipieva simultaneemente Dilantin e riboflavina. Sub iste conditiones, Dilantin habeva nulle effecto. Le patiente remane refractori contra Dilantin un anno post le cessation del administrationes de riboflavina.

Es concludite que Dilantin exerce su effecto toxic in iste patiente per in inhibition specific del synthese de ADN in cellulas erythroide, probabilemente al puncto del formation de deoxyribotida.

Le rolo de riboflavina in le disveloppamento del resistentia contra le effecto toxic de Dilantin in iste patiente remane incerte.

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Biochemical Lesion in Dilantin-Induced Erythroid Aplasia

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