comparable to that achieved *in vivo,* was followed by considerably less product I inactivation (Fig. 2). Loss of product I activity was slow, and even after 30 minutes considerable activity remained. These findings are in conformity with relative stability of product I reported by others(7).

Discussion. Formation of blood thromboplastin is evidently a multi-step reaction. Although the term "product I" is not entirely apt, and its nature is poorly defined, this intermediate can be partially characterized. Its formation depends upon previous activation of a plasma "contact factor" (Hageman factor) and presence of at least antihemophilic factor, Stuart factor, plasma thromboplastin component and Ca^{++}(7). Product I subsequently reacts with phosphatide and factor V to form blood thromboplastin(8). Unpublished studies by Dr. H. Horowitz of this laboratory indicate that reactions leading to formation of product I are slow, but that product I reacts with phosphatide and factor V almost instantaneously to form blood thromboplastin. The slow *in vitro* inactivation of product I here demonstrated is inadequate to account for the rapid restoration of plastic tube clotting time and it may be presumed that its removal from circulation of experimental animals represents a process involving clearance during passage through various organs. Although it appears unlikely that product I rapidly equilibrates with the entire extracellular fluid space, even this degree of dilution would not account for the loss of *in vivo* activity. The present data are not sufficient to suggest which cells or organs are responsible for product I clearance. In previous studies(1) intravenous administration of product I was not followed by increase in circulating anticoagulants. It therefore seems likely that product I is removed rather than neutralized. Rapid removal of product I from circulating blood appears an important component of hemostatic homeostasis, since its prolonged presence would lead to generalized clotting.

Summary. Removal of blood coagulation product I was studied in rabbits. Intravenous injection of this reagent markedly shortened plastic tube clotting time, but restoration to normal occurred in a few minutes. There was no evidence of defibrination. Mixed with plasma *in vitro,* product I was inactivated slowly. It is suggested that product I is removed from the circulation as blood passes through various organs.

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Experimental Epilepsy in the Mouse.* (25889)

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Previous studies demonstrated that a chronic epileptic state may be induced in the monkey (*Macaca mulatta*) by topical or intracerebral application of alumina cream to precentral motor cortex(1,2). Recently, certain pure metals implanted as pellets(3) or powders have also shown epileptogenic properties. Features of monkey epilepsy include spontaneous and induced clinical seizures, lowered thresholds to convulsant drugs(4,5), characteristic electroencephalographic abnor-

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malities and associated focal cerebral lesion at site of application of inciting agent. The present study is concerned with production of a chronic convulsive state in the mouse.

Materials and methods. CF #1 female mice (Carworth Farms) were housed in groups of 4 to 6 in 6 x 10 plastic cages, and maintained on stock diet. At 7-8 weeks of age test material was implanted into brain (scalp incision, light ether) either by stylet through unbeveled tip of 1 mm 23-gauge hypodermic needle or by injection of 0.01-0.02 ml with similar 26-gauge needle. In most instances brains were prepared unilaterally in midsection of cerebrum at 2 sites approximately 3-5 mm from midline. Additional untreated mice were included as controls. Cobalt powder 200 mesh, nickel 325 mesh, antimony 140 mesh were sterilized several hours at 124°C in atmosphere of nitrogen to minimize oxidation. In addition, cobalt was autoclaved for comparative testing. Talcum powder USP, Falba† and aluminum oxide powder were sterilized in autoclave. Falba was emulsified with 2 volumes of saline for injection. Alumina cream was prepared as previously described(2). Post-treatment recovery in most mice was uneventful. Weekly observations were made during cage-cleaning and at random intervals for occurrence of "spontaneous" clinical seizures or those induced by routine handling. Challenge injections of pentamethylenetetrazole (Metrazol)(6) and semicarbazide(7) were selected at dosage levels below the range likely to elicit seizures in normal mice during relatively long experimental period (30 mg/kg subcut. Metrazol 0.3%; 50 mg/kg i.p. semicarbazide HCl 0.5%). Metrazol tests were performed initially at varying intervals after implantation and repeated at approximate 3-week intervals thereafter for 8-10 months. Semicarbazide tests were included toward latter part of experimental period. Control unoperated mice were tested as in treated series. Responses were graded positive when one or more generalized convulsions followed challenge or when only clonic movements of face and forelimbs were noted. Reactions following Metrazol usually occurred 5 to 17 minutes after injection, during 60-minute observation period. Onset of convulsions following semicarbazide appeared 24 to 196 minutes after injection, averaging 73 minutes, during 240-minute observation period. When a series of convulsions followed Metrazol or semicarbazide, amobarbital sodium was administered (50 mg/kg i.p.). Seizures following semicarbazide were further controlled by pyridoxine HCl (50 mg/kg i.p.), which was also administered to negative reactors at end of test period. Procedures of Toman(8) for "psychomotor" seizure test, and of Toman, Swinyard and Goodman(9) for submaximal seizure test were adapted by using shock levels below CD_{50} convulsive threshold for control groups. Rectangular pulses of 1 millisecond duration were delivered through saline-wick corneal electrodes at current values of 5.4-6.4 mA, at frequency of 6/second for 3 seconds for psychomotor test, and 9.5-12.5 mA at frequency of 100/second for 0.33 second for submaximal seizure test (Grass stimulator with oscilloscope). Tests were repeated at intervals of 1 day to 1 week until 2 trials at above mA values were obtained for each method.

Results. Cobalt. Preliminary findings indicated that cobalt powder implanted intracerebrally was an effective epileptogenic agent (occurrence of occasional spontaneous seizures and lowered threshold to Metrazol). To determine onset and duration of susceptibility to Metrazol, 73 mice prepared in either left or right cerebral hemisphere were divided into 7 comparable groups (9-13 mice) and challenged at various intervals. For control, 14 mice were prepared similarly with talcum powder, 12 with Falba and 38 left untreated. Following cobalt implantation (Table I) a lowered convulsive threshold to Metrazol became prominent by second week. At one week only 1 of 10 reacted positively. Between 2 and 10 weeks percentage of positive reactors varied from 40 to 92.3%, with highest initial susceptibility at 4 weeks. Retesting was carried out at 3-week intervals in most instances through 19th week. By this time deaths had

† Pfaltz and Bauer, Inc., N. Y.

TABLE I. Metrazol-Induced Seizures in Mice Following Unilateral Intracerebral Implantation of Cobalt.

Metrazol, 30 mg/kg subcut.			
Initial test		Subsequent tests*	
Wk after implantation	No. pos. / No. tested	Wk after implantation	No. pos. / No. tested
1	1/10	4	4/7
2	4/10	7	16/42
3	5/9	10	17/55
4	12/13	13	23/60
5	4/10	16	22/59
6	7/11	19	16/42
10	6/10	39	14/35
Total	39/73		112/300
Positive	53.4%		37.3%

* 3-5 wk after initial test and at succeeding 3-wk intervals. Controls—Initial test: untreated 0/38, talc 0/14, Falba 0/12. Subsequent tests: untreated 2/324, talc 0/99, Falba 0/91. Additional mice prepared as above with autoclaved cobalt, tested at 5 wk: 5/8 (62.5%) positive.

occurred in 14 mice of the cobalt series (19.2%), 2 of talc-prepared mice (14.3%), none in Falba group (0%) and 2 among untreated (5.4%). Weight increases among surviving animals showed no significant group differences. At 39th week approximately one-half the cobalt group was still alive and susceptible to Metrazol challenge at 40% level. This had remained relatively constant for the group, although mice as individuals did not react consistently on repeated testing. Reactions among controls were essentially negative.

Semicarbazide challenge was postponed until 22-38 weeks after cerebral preparation, when 2 tests were performed at 3-week intervals. No seizures were observed among control groups (11 talc, 12 Falba, 29 untreated). In 52 mice of cobalt series, there were 67 positive reactions to semicarbazide in 104 trials (64.4%). In the same mice, total positive reactions to Metrazol, including 2 further tests after semicarbazide, occurred 191 times in 469 trials (40.7%). When cobalt-treated mice were arranged according to individual scores to Metrazol challenge into: (a) those that were positive under one-third of times tested (24 mice) and (b) those positive over one-third of times tested (28 mice), positive reactions to semicarbazide challenge for the groups were: (a) 22/48 or 45.8% and (b) 45/56 or 80.4%.

Electroshock tests were performed following final Metrazol challenge in mice described above, the series including 41 cobalt-treated and 49 control mice (11 talc, 11 Falba, 27 untreated). In the psychomotor test, symptoms of minor seizures similar to those described by Toman, as well as "submaximal" seizures, were observed in 22 cobalt mice, and minor seizures alone in 11 controls. The difference between cobalt-treated and control group was significant ($p<.01$), and thus suggests a lowered psychomotor seizure threshold in cobalt-treated series.

When submaximal seizure test was applied to mice of the same groups, the reactions observed presented features characteristic of submaximal seizures in 8 of 41 cobalt-treated animals and in 1 of 49 controls. The difference between groups was significant ($p<.02$). This test also elicited reactions characteristic of minor seizures in 11 cobalt-treated and 3 controls, all but one of which (cobalt) failed to show submaximal seizures. This difference between groups was also significant ($p = .02$). The findings indicate therefore that cobalt treatment had lowered the threshold for submaximal and minor responses.

No routine attempt was made to elicit seizures by stressful prodding (as in experimental monkey epilepsy), to avoid possible effects on challenge with the convulsant drugs. "Spontaneous" seizures were distributed over entire test period and represented a minor proportion of all recorded convulsive reactions (58 spontaneous). No similar seizures were seen among control groups.

Nickel. Twenty-one mice received cerebral implantations of nickel powder either unilaterally or bilaterally. Few spontaneous clinical seizures were observed. Initial Metrazol challenge at 3 weeks proved positive in 5 of 12 prepared unilaterally (41.7%) and in 2 of 9 prepared bilaterally (22.2%). Subsequent retesting at 6-9, 12-19, 20-32, and 39-42 weeks (cumulative results) yielded positive group averages varying from 33.3 to 38.9% (unilateral) and 22.2 to 38.9% (bilateral). In 2 trials with semicarbazide 8 of

TABLE II. Production of Epilepsy in Mice by Intracerebral Implantation of Cobalt and Nickel.

Treatment	Metrazol,* 30 mg/kg subcut.			Semicarbazide,† 50 mg/kg i.p.		
	No. of mice	% mice positive	% tests positive	No. of mice	% mice positive	% tests positive
Cobalt	73	86.3	42.7	52	75.0	64.4
Nickel	21	85.7	33.8	14	57.1	42.9
Falba	21	0	0	16	0	0
Talc	25	0	0	24	0	0
Untreated	38	4.9	.6	30	0	0

Bilateral preparations: Nickel 9, Falba 9, talc 11.

* 1 to 10 trials over 10 mo period. † 2 trials on survivors 6-8 mo after preparation.

14 mice (4/7 unilateral, 4/7 bilateral) reacted positively (57.1%). Totals are included in Table II.

Antimony. This metal proved too toxic when applied to the brain as used for cobalt and nickel. Most mice so treated died within a few days to a week, although occasional survivors showed lowered convulsive threshold to Metrazol challenge. To prepare mice, therefore, antimony powder was mixed with talc (1:3, 1:7 or 1:15 by weight) before cerebral implantation. Of 11 mice so treated, 5 were positive to Metrazol on at least one trial. Five survivors at 38-44 weeks tested negative to semicarbazide.

Alumina cream was of especial interest because of its high degree of effectiveness in inducing epilepsy in the monkey. Numerous attempts to adapt its use to the mouse, employing 1 to 4 injection sites, unilaterally or bilaterally, as well as various dilutions of the suspended sediment, resulted in only occasional animals with spontaneous clinical seizures or lowered convulsive threshold to Metrazol. Implantation of aluminum oxide powder yielded negative results.

Focal nature of reaction. Clonic seizures confined to one side of body were observed in 14 mice prepared with cobalt unilaterally. Of these, 5 occurred spontaneously and all were contralateral to the prepared cerebral site. Of those reactions which followed Metrazol activation, 8 of 9 were contralateral to the cerebral site.

Discussion. Recurrent convulsive seizures in mice have certain features in common with those previously noted in experimental epilepsy in monkeys. These include incubation period following cerebral implantation of inciting agent, contralateral focal reactions, generalized tonic-clonic seizures, and lowered thresholds to convulsant drugs. Criteria for epilepsy in the mouse have been confined in our report to clinical seizures induced by challenge with Metrazol, semicarbazide and electroshock, at levels below convulsive thresholds for normal animals. Our findings are consistent with the knowledge that while the epileptogenic lesion (man) itself is chronic, clinical seizure discharges may be intermittent.

Use of the mouse as chronic experimental epileptic animal should prove of value in neurophysiologic, biochemical, pathologic and other studies, including screening and evaluation of anti-epileptics, as well as for investigations of effects of pharmacologic agents on the central nervous system.

Summary. Chronic epilepsy has been produced in the mouse by cerebral implantation of various powdered metals, notably cobalt and nickel. In addition to spontaneous focal and generalized seizures, epileptic status was evaluated by response to challenge injections of Metrazol and semicarbazide, as well as to electroshock.

Addendum. Cobalt implanted intracerebrally, in the manner described for mice, has recently proved highly epileptogenic in the rat as evidenced by lowered convulsive threshold to Metrazol.

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Effects of Administration of L-Thyroxin on Liver N-Demethylating Activity in Normal and Morphine-Treated Rats. (25890)

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Chronic administration of morphine to rats causes a striking diminution in ability of liver microsomal-supernatant preparations from these rats to N-demethylate narcotic drug substrates(1,2). After abrupt withdrawal of drug from rats treated with morphine chronically, complete recovery of this enzyme activity occurs within 12 days(1,3). Recently L-thyroxin stimulated incorporation of amino acids into protein(4), suggesting that it accelerates protein synthesis. Since recovery of N-demethylase activity after withdrawal of narcotic drugs may represent enzyme resynthesis, it was of interest to study the effects of thyroxin on this process.

Methods. Forty-four NIH male black rats, each weighing 200-300 g, were divided into 4 groups so matched that average weight of each group was the same. The 4 groups were treated as follows. For 30 days, Group M received intraperitoneal injections of morphine sulfate in gradually increasing doses from 20 mg/kg to 100 mg/kg twice daily. Group MTh received morphine in same amounts and on same schedule as Group M and, in addition, a daily intraperitoneal injection of 90 μg of L-thyroxin in 0.5 ml of 0.01 N NaOH for 7 days prior to abrupt withdrawal of morphine and throughout period of withdrawal until day of sacrifice. Group Th received only thyroxin in same amounts and on same schedule as group MTh. Animals in group C were untreated controls. At 19 hours, 96 hours, and 8 days after termination of morphine administration, animals from the 4 groups were sacrificed and their livers prepared for enzyme assay by method of Axelrod(5). Enzymatic activity was measured by estimating the amount of formaldehyde formed by the N-demethylation of morphine, as described previously(2).

Results of representative experiment are summarized in Fig. 1. Nineteen hours after withdrawal of morphine and 7 days after initiation of thyroxin treatment in groups receiving the hormone, the N-demethylation of morphine by liver preparations from animals in group M was 6.7% of untreated-control value, and the activity in preparations from animals in group MTh was about one-half that of group M ($p<.01$). Preparations from animals receiving L-thyroxin alone for 7 days did not differ in N-demethylase activity from untreated controls. By 96 hours after withdrawal (and after 10 days of thyroxin administration in thyroxin-treated animals) N-demethylating activity began to recover in both groups M and MTh, and no significant difference between them was observed ($p>.05$). Then, a marked depression of activity was observed in animals treated with thyroxin alone (group Th). Eight days after morphine withdrawal, enzymatic activity of liver preparations from rats in group MTh was still less than one-fourth of control preparations (Group C) and had not increased appreciably from values obtained previously. On the other hand, N-demethylase activity for animals in Group M showed at this time an almost complete return to normal. Administration of thyroxin for 14 days had depressed