Reports and Letters

Interaction of Molybdenum and Iron in Soils

The availability to plants of molybdenum in some acidic soils may be increased by raising the *p*H of the soil. This relation to *p*H is analogous to that of phosphate which is held in insoluble form by iron and aluminum, and it suggests a study of the interactions of molybdate with iron and aluminum. This paper (*1*) is concerned with interactions with iron in simple systems of ferric oxide as well as in soils.

A ferric oxide, which was amorphous to x-rays, was prepared by the method of Schuylenborgh and Arens (*2*) and ground to pass a 200 I.M.M. sieve. Solutions of sodium molybdate containing 100 μg of Mo were added to 100-mg samples of ferric oxide. The *p*H's of the suspensions were adjusted, and the total volume of each was brought to 50 ml. After mechanical shaking of the solutions for 15 hours, the *p*H's were determined, the suspensions were centrifuged, and the Mo remaining in solution was determined (*3*) by the colorimetric-dithiol method of Piper and Beckwith (*4*). The ferric oxide removed the following amounts of Mo from solution: 100 μg at *p*H 4, 100 μg at *p*H 5, 100 μg at *p*H 6, 98 μg at *p*H 7, 83 μg at *p*H 8, and 22 μg at *p*H 9.

In further experiments with ferric oxide, the amount of Mo adsorbed was determined as a function of the equilibrium concentration of Mo in solution for equilibrium *p*H 4.5. The adsorption isotherm had a high initial gradient and, at an equilibrium concentration of 50 μg of Mo in 50 ml of solution, it leveled off with a saturation value of about 7 mg of Mo per 100 mg of solid. An aluminum oxide (Böhmite) prepared by the method of Schuylenborgh (*5*) was found in similar experiments to be saturated with 3.5 mg of Mo per 100 mg of solid. The difference between the initial gradients was more marked, that for ferric oxide being about 50 times greater than that for aluminum oxide. The effectiveness of the clay minerals in adsorbing molybdate was less than that of the sesquioxides and decreased in the order halloysite, nontronite, and kaolinite.

Table 1. Molybdenum adsorbed (μg) from a total of 500 μg by 5-g samples of soils.

Soil	*p*H 4.0	5.0	6.0	7.0	8.0
Wollongbar, untreated	500	498	460	270	80
Wollongbar, minus Fe_2O_3	425	340	190	45	0
Elmhurst	450	310	100	20	0

The fact that ferric oxide adsorbs so much molybdate led to experiments with two soils that were, respectively, high and low in colloidal ferric oxide. The first soil was a krasnozem of *p*H 5.3 from Wollongbar, near Lismore in New South Wales. It contains 55 percent clay (particle size less than 2 μ) and 14.9 percent free ferric oxide. The other soil, from Elmhurst, near Ararat in Victoria, was a grey, gravelly loam of *p*H 5.9, overlying yellow clay at 10 in. It has 14 percent clay (particle size less than 2 μ) and 1.1 percent free ferric oxide.

The soils were air-dried and ground to pass a 60-mesh I.M.M. sieve. Samples of 5 g were shaken for 15 hours at adjusted *p*H's with solutions containing 500 μg of Mo in a total volume of 50 ml. After the suspension had been centrifuged, the Mo remaining in solution was determined as before. Parallel experiments were performed on 5-g samples of the Wollongbar soil after the free ferric oxide in them had been removed by Jeffries' method (*6*). In this method, the iron oxide is reduced by nascent hydrogen in oxalic acid, and the iron is then removed in solution.

The results (Table 1) show again that the greatest amounts of molybdate were adsorbed at low *p*H's, and that the amounts adsorbed decreased with increasing *p*H. The untreated Wollongbar soil adsorbed much more molybdate than the Elmhurst soil, and even at *p*H 7, the Wollongbar soil still adsorbed approximately half of the molybdate presented. The effect of removing the ferric oxide from this soil was striking. The amounts adsorbed were thereby reduced to amounts nearer those adsorbed by the Elmhurst soil. Thus, at *p*H 6, the adsorption by the Wollongbar soil was reduced from 460 μg to 190 μg, the amount adsorbed by the Elmhurst soil being 100 μg.

The importance of ferric oxide in soils is shown both by the relatively large amounts of molybdate that it adsorbs in simple systems and by the effect of removing it from the Wollongbar soil. Since adsorption is greatest in acidic systems and becomes less with increasing *p*H, it is suggested that Mo, in ferruginous soils, is held on the surface of colloidal ferric oxides as the molybdate anion, which is replaceable by hydroxyl ions.

This work fits in with the effect of lime on the availability of Mo to plants on some acidic, ferruginous soils and also with the principle used by Grigg (*7*) in his analysis for available Mo in soils—namely, that an amount of Mo which is sufficient for plants at *p*H 6 may be insufficient at *p*H 5. Another piece of compatible evidence is that presented by Williams and Moore (*8*) who, by means of chemical analyses of plants and soils, arrived at an equation relating Mo in plants to both *p*H and iron of soils. Their equation is of the form

$$\log \mathrm{Mo} = a\ p\mathrm{H} - b\ \mathrm{Fe} + c,$$

where the Fe was that dissolved by boiling the soil with 6*N* hydrochloric acid.

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6 February 1956

Effect of Reserpine on Learning and Performance

Reserpine, an alkaloid extract of *Rauwolfia serpentina,* is now widely used in clinical psychiatry. It has been shown that it depresses well-established performance patterns of rhesus monkeys—for example, pressing a bar to avoid shock or to obtain food (*1*). The present study shows that reserpine can depress "discriminated" or "conditioned" responses, as well as "operant" responses, and suggests that reserpine depresses learning as well as performance.

The technique employed was the establishment and extinction of a conditioned emotional response to a signal (white noise) that preceded a noxious stimulus (electric shock). We observed the caged animal from an adjoining room through a one-way window, and independently rated responses to the noise and shock on a four-point scale. A rating of "zero" indicated no response, "one" a questionable response, "two" a fairly definite response, and "three" a very definite response. Response was defined as any recognizable change in ongoing behavior. The various responses to the sound included running, crouching, climbing, and lying prone. The pattern of response most commonly conditioned to the sound was a period of running, followed by crouching.

Eight rhesus monkeys, approximately 2 years of age, served as subjects. They were first given eight preconditioning trials consisting of the noise alone. The noise was approximately 25 decibels sensation level (human) and was sounded for 20 seconds on each occasion. The intervals between successive presentations of the noise were randomized, with an average interval of 2 minutes. The following day the monkeys were divided into two groups of four members each, a reserpine-conditioning group and a saline-conditioning group, and given the appropriate injection. A dosage of reserpine was selected (0.75 mg/kg) that is within the range of previous studies in which monkeys were employed and that typically depresses the general behavior of the animal significantly (*1, 2*). The saline dosage chosen (0.3 ml/kg) was the same in volume as the reserpine solution.

Conditioning was begun 3 hours after injection for the reserpine group. The interval between injection and conditioning for the saline group was of the same order, but it was not carefully controlled. In the conditioning procedure, each presentation of the noise was followed by five short, strong pulses of electric shock (one per second) delivered by a method described elsewhere (*1, 3*). The injections and conditioning procedures were repeated 2 days later. All animals received ten trials on each of the 2 days.

Three days after the second conditioning day, all animals were tested (without further injections) for "retention" of the conditioning experience. Conditioning trials were presented to a given animal at the rate of ten per day on alternate days, until a series of five successive noise responses was obtained, such that both investigators gave ratings of "two" or "three" to any four of the five responses.

Table 1. Conditioning

Conditioning retention		Conditioning
Reserpine (1)	Saline (2)	Saline (3)
18	0	9
13	0	7
7	0	7
2	0	3

In the study of extinction, the eight animals were again divided into reserpine and saline groups of four members each. The reserpine-extinction group consisted of two members from the reserpine-conditioning group plus two members from the saline-conditioning group, while the saline-extinction group consisted of the remaining two members of each of the two conditioning groups. Injections were given before each session. In the extinction procedure, electric shock was not presented. The trials were given in two sessions of ten each and were separated by 2 days. Three days later, the animals were tested for "extinction retention" by presenting further extinction trials (without injections), ten per day on alternate days, until a series of five successive noise responses was obtained, such that both investigators gave ratings of "zero" or "one" to any four of the five responses.

The following results were obtained. During conditioning, the reserpine group showed only slight deviations from "zero" values in their responses to the noise, although they definitely responded to the electric shock. The saline group showed a definite increase in noise response values. The following figures are the averages of both our ratings for each group of animals during the first ten and the second ten conditioning trials (the numbers in parentheses represent the range of the average ratings for each trial): reserpine group—0.03 (0 to 0.2), 0.17 (0 to 0.4); saline group—1.70 (0.9 to 2.6), 2.40 (1.9 to 2.8).

In Table 1 is listed the number of trials (not including the criterion trials) required for each animal to reach the retention criterion. All saline animals had perfect retention (column 2), while all reserpine animals required additional training (column 1) (this difference is significant at the 0.05 level by the Mann-Whitney test). Column 3 lists the number of trials in which the saline animals achieved criterion performance, if computations are made from the beginning of the conditioning period. This is a measure of how long it took control animals to learn this particular habit. By comparing the reserpine retention scores with the scores in column 3, one can determine whether the reserpine animals benefited from their experience under the drug. It can be seen that the difference between column-1 and column-3 scores is not significant, although the reserpine mean (and variance) is slightly larger.

In extinction, the reserpine group again showed considerably lower noise-response values than the saline group, regardless of which drug had been used during conditioning. The average ratings and ranges for the first ten and second ten extinction trials were as follows: reserpine extinction-reserpine conditioning group—0 (0 to 0), 0.05 (0 to 0.5); reserpine extinction - saline conditioning group—0.05 (0 to 0.5), 0.20 (0 to 0.5); saline extinction-reserpine conditioning group—1.75 (1.0 to 2.0), 1.70 (1.0 to 2.0); saline extinction-saline conditioning group—2.15 (1.8 to 2.5), 1.71 (1.0 to 2.5).

The first two columns of Table 2 give retention scores. The response of the animals that had had conditioning with saline was extinguished more slowly than the response of the animals that had had reserpine conditioning, regardless of which extinction drug was employed—this is presumably further evidence that the saline animals had learned more during the conditioning period. The reserpine extinction animals required more trials, on the average, to reach criterion than did the saline animals. Indeed, there is almost no overlap between the extinction subgroups. The reserpine group required slightly fewer trials, on the average, for extinction during the retention period than the total number of trials that the saline animals required during both the extinction and extinction retention periods (column 3).

Table 2. Extinction

	Extinction retention					
Extinction drug	Reserpine (1)		Saline (2)		Extinction Saline (3)	
Conditioning drug	Reserpine	Saline	Reserpine	Saline	Reserpine	Saline
Trials	12	40	0	14	20	34
Trials	10	13	0	2	19	22

On the present evidence, the most parsimonious interpretation is in favor of reserpine's depressing temporarily both performance and learning—that is, that the drugged animals were functionally impervious to conditioning and extinction events, had to "start from scratch" once the drug had worn off, but subsequently responded normally to such events. Insofar as the reserpine groups, when tested after the gross effects of the drug had dissipated, differed from the controls in their rate of conditioning or extinction, they required more conditioning trials and fewer extinction trials, although these differences are far from being statistically significant. If, with a larger N or more refined technique, such differences were to become significant, explanation might follow one of several courses.

Examples of such possible explanations include the following: slight amounts of reserpine (or a metabolic product) might be active in the organism long after its gross effects had disappeared; in extinction, the reserpine animals have a longer time to "forget" the conditioned response, if they are impervious to the extinction events; the "baseline level of anxiety" might remain lower even after the drug has been completely metabolized.

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13 December 1955

New Theory of Interference in Clotting Mechanism by Abnormal Plasma Proteins

The present communication postulates a new type of defect in the clotting mechanism. This consists of the production by the body of globulins that have the ability to combine with and precipitate prothrombin and accessory factors from the blood plasma. The consequence is that the normal clotting mechanism is unbalanced. This results in the following changes: (i) a reduced concentration of clotting factors in the circulating blood, causing hemorrhages, and (ii), localized concentrations of prothrombin and accessory factors, causing thrombi.

Clotting defects, both hemorrhages and thromboses, are known to occur in association with clinical conditions in which abnormalities in the plasma globulins are well recognized, such as macroglobulinemia (*1*), multiple myeloma (*2*), purpura hyperglobulinemia (*3*), and cryofibrinogenemia (*4*). In addition, there exist clinical conditions such as carcinomatosis (pancreatic and so forth) (*5*), thrombophlebitis (*5*), pulmonary infarction, coronary occlusion, abdominal thrombosis, and postoperative thrombosis in which the causes of the thromboses have been the subject of investigation for many years without elucidation of their etiologies. Such cases now require reinvestigation in the light of our present theory.

This theory is based on the demonstration (*6*) that the precipitation of euglobulin, by dilution of plasma, takes out with it prothrombin and factor VII (stable factor). In one case (L. S.), the euglobulin consisted of macroglobulins with a major ultracentrifuge sedimentation component at $S_{20} = 20$ and two minor components at $S_{20} = >20$. In a second case (R. B.), euglobulin and cryoglobulin precipitates were obtained, both of which precipitated out prothrombin and accessory factors (factor VII), the euglobulin to a greater extent than the cryoglobulin. The euglobulin contained no macroglobulins, contrary to expectations. Both cases had increased plasma viscosity, which showed an anomalous rise with decreased temperature. Cryoprecipitability could be elicited in one case (R. B.) by prefreezing the plasma sample and in both cases by reducing the salt concentration of the sample. Macrogobulin precipitated under these conditions (L. S.) can be redissolved by the addition of albumin or minute traces of sodium carbonate but not by gamma globulin.

Both cases had hemorrhagic tendencies and reduced prothrombin activity. Case L. S. also had an extensive thrombus of the iliac vein. Precipitation of the euglobulins from both cases caused a marked reduction of both prothrombin and factor VII in the euglobulin-free plasma. The prothrombin and factor VII could be demonstrated in the solution of the precipitated globulins. The addition to and precipitation of macroglobulin from normal plasma removed prothrombin and factor VII from the normal plasma.

In addition to the afore-mentioned results, we have found that cold precipitable material is obtainable from normal and pathological plasmas. This material contains both fibrinogen and small amounts of prothrombin. Hence, the cold precipitation of fibrinogen takes down with it prothrombin. Studies of cryoglobulins are in progress. It is highly probable that prothrombin combines easily with many globulins in addition to the accessory factors required in the clotting mechanism. The ease with which prothrombin is adsorbed by barium sulfate is well known. Slight changes in the plasma globulins that permit precipitation, therefore, would sequester prothrombin from the circulating plasma.

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6. This work was aided by a grant from Leukemia Research, Inc., Los Angeles, Calif. A more detailed report is in preparation.

6 February 1956

Pollen from Leda Clay of Maine

Marine clays of late glacial age occur widely in eastern Maine from below sea level to 400 ft or more above. These clays occasionally contain a well-preserved molluscan fauna of northern affinities, suggesting that, during the time of their deposition, the water was colder than it is at present. Because of the presence of species of *Leda,* these clays are frequently referred to as Leda clays, although their affinity is closer to the later *Saxicava* phase of the marine sediments in southeastern Canada.

One of the most productive fossil localities of the Leda clay in Maine occurs at Goose Cove on Mount Desert Island. The fauna from this locality has been described by Blaney and Loomis (*1*), who note that the assemblage has strong affinities with the present molluscan fauna of Labrador.

I had occasion to examine the clay from this locality for microfossils. The sample from which the analysis was made came from highly fossiliferous beds about 10 ft above high tide near the head of the cove. The microfossils were separated by a bromoform-acetone mixture (*2*) with a specific gravity of 2.3. A large number of pollen grains and spores, together with some marine and brackish-water sponge spicules and a few Foraminifera, were recovered. Table 1 shows the result of the pollen-spore analysis.

The pollen content of the clay is very similar to the pollen assemblages that I found in late glacial clays of southeastern New England, but it differs in several