

Influence of Salicylate Administration on Iron Metabolism

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SEVERAL PAPERS have appeared lately in which evidence was brought forward that the administration of salicylates may cause blood loss from the gastrointestinal tract, and thus might produce iron deficiency anemia.¹⁻³ The present study was carried out to investigate the role of salicylates in the causation of this anemia and to test the possibility that salicylates, in addition to their effect on the mucous membranes of the stomach and intestines, might otherwise interfere with the metabolism of iron. One of the ways to approach this problem seemed to be a study of the behavior of the serum iron during salicylate administration. The results of such investigations are reported in this paper.

MATERIAL AND METHODS

Eleven subjects were included in this study. In none of them was there evidence of a disease of the hematopoietic system, and in only one of them was there any known lesion of the gastrointestinal tract. Two patients (Cases 6 and 11) were slightly anemic; nine of the subjects were patients and two were normal healthy volunteers. The patients were hospitalized during most of the observation period, whereas the healthy subjects continued their regular medical work. In all subjects a careful history was obtained and routine physical and laboratory examinations performed.

A number of hematologic examinations were done before, during and after the administration of salicylate. These included hemoglobin, hematocrit, red blood cell and reticulocyte counts (1000 cells were counted), total and differential white blood cell counts by the usual methods. In addition, serum iron and unsaturated iron binding capacity⁴ were determined in all subjects. Fe⁵⁹ absorption, utilization and excretion studies were performed as described by Dubach and associates⁵ on three subjects. In one patient (Case V) Fe⁵⁹ absorption and utilization studies were performed before salicylate administration and repeated three weeks later while he was taking the drug. In a second patient (Case 6), this study was performed during salicylate administration only.

Cr⁵¹ tagged red cells were re-injected intravenously into three of the patients (Cases 7, 8 and 11) while on salicylates, and stool samples were collected over a period of at least one week. The total radioactivity in the daily stool samples was determined and the amount of blood in it calculated.⁶ The survival of the tagged cells was also determined in these three patients.⁷

Salicylate levels in the plasma were determined in random blood samples in all subjects during the administration of the drug.†

Procedure

The patients were given the regular hospital diet; the normal subjects took a normal varied diet in their own homes. Two or three complete blood counts, serum iron and iron

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*Five μ C Fe⁵⁹ was administered together with 25 mg. metallic iron as carrier.

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binding capacity determinations were performed in the course of 3–6 days before the salicylate was administered. The drug was given in the form of acetylsalicylic acid (aspirin) in 0.5 Gm. tablets over a period varying from one to four weeks in doses of 40–60 mg./Kg. daily. No other drugs were administered during this time. Twice weekly, whole blood counts, serum iron and unsaturated iron binding capacity determinations were performed during the salicylate administration, and, in seven of the subjects, also for periods varying from two to six weeks after the administration of the drug was discontinued. As some patients were discharged home during this last period, and as some of them had been in the habit of taking analgesics, the possibility that aspirin was again taken occasionally could not be excluded in two of these seven patients (Cases 1 and 6).

The procedure in the normal subjects was somewhat different from that described above. In view of the possibility that the changes in serum iron and hemoglobin values observed in the patients during the salicylate administration were not due to a metabolic effect of the drug, but to blood loss, either from the gastrointestinal tract or through repeated venipunctures for the necessary estimations, the administration of aspirin was preceded by a period in which blood in amounts of 15–30 ml. was drawn regularly, until a total of 135 ml. in two weeks was taken from the veins in these subjects. Thus the effect of repeated blood drawings could be studied separately. Only after this procedure was the salicylate administration started.

Control Experiments in Vitro

To rule out the possibility that the changes in serum iron observed during this study were due to interference of salicylates with the method of determination, the serum iron content was estimated on duplicate samples by the method regularly used,⁴ and by the method of Moore et al.⁸ in which the serum iron is determined after treatment of the serum sample with HCl and subsequent charring of the protein.

Other controls to exclude the possibility of in vitro effects were carried out by incubating plasma obtained from normal individuals (after they had been given iron intramuscularly, so that they had a high plasma iron concentration) with varying concentrations of aspirin or sodium salicylate, following which the iron content of the plasma was determined. In some experiments, salicylate containing plasma (obtained from patients given aspirin by mouth) was incubated with plasma rich in iron from normal subjects, after which the iron concentrations in the incubation mixture were again determined.

RESULTS

Serum Salicylate Levels

These were determined on several samples during the administration of aspirin. There was some fluctuation of the values found but in general they were of the same magnitude as commonly encountered in patients who are being treated with salicylates for therapeutic purposes, viz. 14–35 mg. per cent.

The Effect of Salicylate on Serum Iron Determination in Vitro

Table 1 shows the results of the experiments in which salicylate, or plasma from a patient receiving salicylates, was incubated with plasma rich in iron content. (No effort was made to determine whether the circulating iron was attached to dextran or to the physiologic iron binding protein.) Following incubation there was no decrease in the amount of iron demonstrable in the plasma. The slight variations in the values obtained are within the range of error of the methods employed. Table 1 also contains the results of a comparison of the determinations of serum iron without and with prolonged extraction

Table 1.—Incubation Mixtures—Summarizing Control and Recovery Experiments

Exp. No.	Plasma \bar{c} Salicylate* ml.	Plasma \bar{c} Iron† ml.	Salicylate Dissolved in H ₂ O 240 mg. %	Iron-Free H ₂ O ml.	Following Incubation		Remarks
					Iron γ %	Recovered %	
1	2	—	—	—	89	100	
2	—	2	—	—	376	100	
3	1	1	—	1	149	97	
4	1	1	—	1	155‡	100	
5	0.5	1	—	1.5	151	107	
6	0.5	1	—	1.5	151‡	107	
7	1	0.5	—	1.5	100	108	
8	1	0.5	—	1.6	98‡	106	
9	—	1.8	0.2	—	300	89	
10	—	1.8	0.2	—	315‡	93	
11	—	2§	—	—	48	100	
12	—	1.8§	0.2	—	44‡	102	Sodium salicylate used
13	—	1.8§	0.2	—	41	95	Sodium salicylate used

*Plasma obtained from patient given salicylate over a period of one week previous. At the time of the experiment, plasma salicylate concentration was 35 mg. per cent.

†Plasma obtained from patient given iron prior to experiment.

‡Iron determination performed according to the method of Moore et al.⁸

§Plasma obtained from patient with iron deficiency anemia following salicylate administration.

and charring on duplicate samples taken from one of the patients while he was taking salicylates. It is evident that even while salicylates were present in the plasma, there was good agreement between the two methods.

Serum Iron Levels

In every subject given salicylate, a drop in the serum iron level occurred as compared to the control values. The decrease in the average values before and during administration ranged from 11–61 per cent; the maximum drop ranged from 35–78 per cent of the original serum iron concentration. The phenomenon was highly significant statistically ($P < 0.01$). The drop started to occur on the 2nd to 7th day and became maximal between the 9th and 36th day after the administration of aspirin was instituted. After the salicylate administration was discontinued, the serum iron rose again in most cases but in only one of the eight subjects (seven patients and one normal) in whom we followed its course did it reach its original level again. This is illustrated in table 2 and figures 1–3.

Unsaturated Iron Binding Capacity

The effect of aspirin administration on the unsaturated iron binding capacity of the serum was more irregular than on the serum iron. In general, considerable fluctuation occurred both during and after the administration period. In eight cases there was an initial drop in the unsaturated iron binding capacity, which occurred between the second and seventh day of the administration; in four of

Table 2.—Survey of Average Values for Serum Iron, Hemoglobin, Hematocrit, and Reticulocytes Before, During and After Administration of Salicylates

Case No.	Diagnosis	Sex	Serum Iron γ %	Hemoglobin Gm. %	Hematocrit (%)	Reticulocytes (%)
1	Essential hypertension	male	89-79-84	15.2-14.7-14.7	48-46-44	0.6-1.2-0.8
2	Rheumatic heart disease	female	140-55-86	15-12.2-11.9	46-40-41	0.5-0.8-0.8
3	Diverticulosis of colon	male	126-82-109	14.1-13.8-14.2	47-43-47	1.0-0.9-0.5
4	Essential hypertension	female	153-72-79	13.6-12.7-12.4	44-41-41	0.4-0.9-0.6
5	Atherosclerotic peripheral vascular disease	male	138-98-88	14-13.1-12.5	43-42-41	0.4-0.7-0.6
6	Osteoarthritis of spine	female	63-45-46	12.2-11.2-11.0	42-38-37	0.7-0.9-0.9
7	Neurofibroma of cauda equina	female	119-59	14.2-13.2	44-42	1.0-2.0
8	Essential hypertension	male	90-60-66	14.7-14.3-14.0	47-45-42	2.0-0.3-0.8
9	Normal subject	male	110-62-85	15.1-14.9-14.8	47-46-45	0.6-1.2
10	Normal subject	male	123-82	14.8-15.2	47-48	0.3-0.7
11	Rheumatoid arthritis	male	71-38	12.4-12.0	41-40	1.2-2.2

the cases this was followed later by a rise above the original value (figs. 1-3). These changes, however, were not statistically significant.

Blood Count

In all the patients there occurred a drop in hemoglobin, usually small but significant ($P < 0.01$); after the salicylates were discontinued the hemoglobin rose slightly but the average values did not return to the original level and in several cases either remained the same or showed a (non-significant) decrease compared to the experimental period. The hematocrit values also decreased significantly during the administration and also remained low after the drug was stopped. There was no significant change in the erythrocyte counts, either during or after the administration.

Fe⁵⁹ Absorption, Utilization and Excretion

In case 5, in whom the first absorption study was performed before salicylate was started, 35 per cent of the administered dose was absorbed and the peak of activity in the RBC occurred between the seventh and tenth day following the administration of Fe⁵⁹, which is normal. The same study was repeated three weeks later when the patient took salicylate; then 39 per cent of the administered iron was absorbed from the G.I. tract, but the peak in the RBC occurred on the sixth day after the radioiron administration, showing a steeper rise during the days prior to this sixth day. This type of curve is observed in our laboratory in patients with hypochromic iron deficiency anemia. A similar pattern of absorption and utilization curve was obtained in case 6 who was given the Fe⁵⁹ only during salicylate administration (figs. 4-5). Nor was there a secretion of Fe⁵⁹ of any magnitude in the urine either before or during salicylate administration.

Blood Loss Through the G.I. Tract

In case 7 a Cr⁵¹ blood loss determination (fig. 6) showed a total loss of 30 ml. of blood over a period of 15 days while the patient was given salicylates. A similar test performed on case 8 disclosed a total loss of 13 ml. in the stools over a one week period during the salicylate administration. The third patient

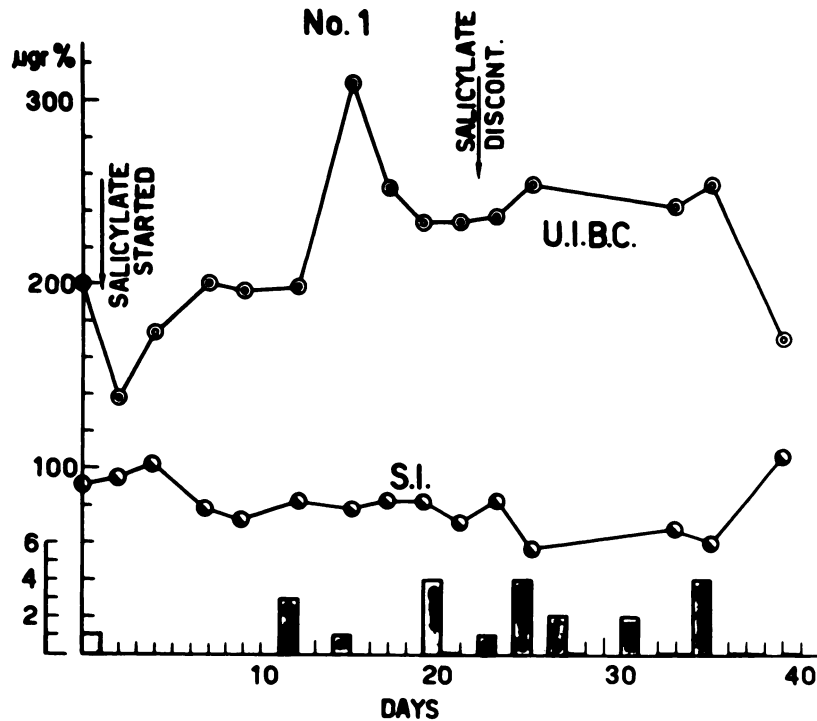


Fig. 1.—Case No. 1. Decrease in serum iron and irregular behaviour of unsaturated iron-binding capacity during the administration of salicylate. Positive benzedine tests in the stools are indicated by black blocks above the abscissae.

(case 11) appeared to be losing occult blood before salicylate was administered in a quantity of 26 ml. over a period of 10 days. Continuation of the test during the salicylate administration indicated a blood loss of 61 ml. during a period of 10 days.

The Effect of Repeated Blood Drawing on Serum Iron and on Blood Count

While repeated bleedings totaling 135 cc. of blood taken from the two normal subjects in two weeks had no or very little effect on their blood count and serum iron levels, a sharp decrease in serum iron promptly followed the administration of salicylates in both subjects. This result can be seen from figure 7.

Red Blood Cell Survival

In the three patients (7, 8 and 11) in whom the survival of red cells was determined, there was a decrease in half-life of the Cr^{51} tagged red cells to 21, 21 and 20.5 days respectively (compared to the normal of 30 days).

DISCUSSION

The most immediate and constant result of the salicylate administration was a drop in serum iron, which was observed in all cases. However, a considerable variation appeared to exist both in the time of onset and the magnitude of

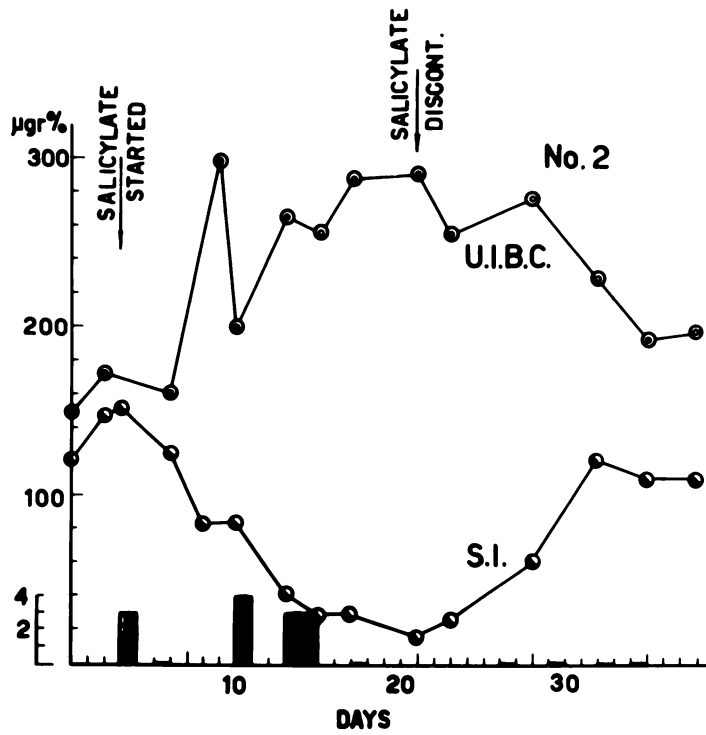


Fig. 2.—Case No. 2. For legend, see figure 1.

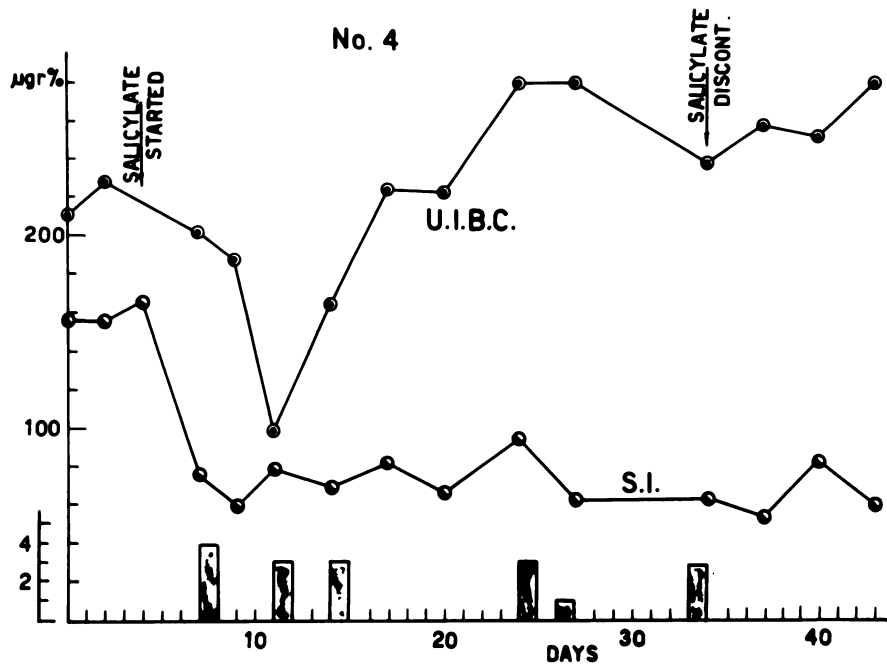


Fig. 3.—Case No. 4. For legend, see figure 1.

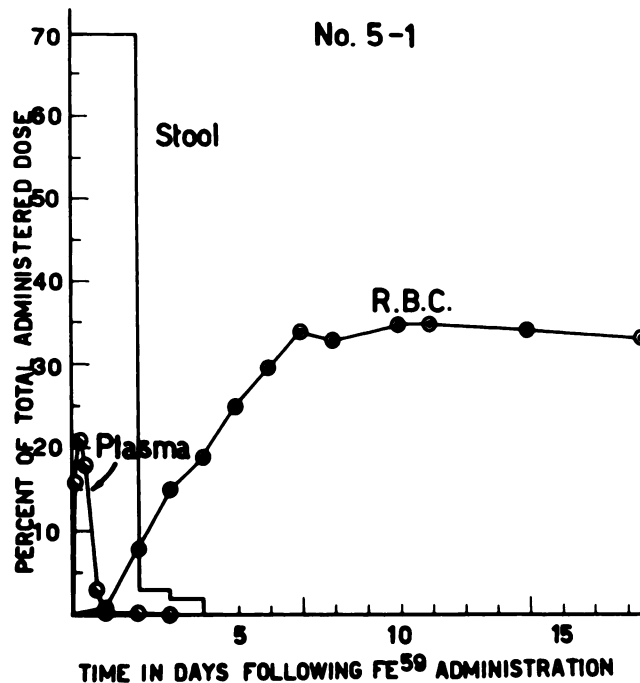


Fig. 4.—Case No. 5. Absorption of Fe^{59} as shown by appearance of radioactivity in plasma and excretion in stools; utilization of Fe^{59} as manifested by its appearance in the red blood cells. Test was carried out before salicylate was started; absorption and utilization are normal.

this drop. In searching for the mechanism responsible for this decrease in serum iron, one had to consider first the possibility that the salicylate or its metabolites in the plasma might interfere with the determination of the serum iron. This possibility was ruled out by the control experiments described above.

Two other possibilities had to be considered in this respect, viz., that the drop in the serum iron was caused by blood loss through venipunctures, necessary to obtain blood samples for the laboratory examinations, and/or through blood lost into the gastrointestinal tract. A number of papers have appeared during recent years, in which it has been shown that individuals who receive salicylates lose blood in their stools, as evidenced by a positive benzedine test and by spectroscopic methods.^{1,2}

In trying to evaluate the possible role of the blood loss in the decrease of serum iron, the amount of blood lost along the gastrointestinal tract was measured in three patients after re-injection of Cr^{51} labeled erythrocytes. These measurements revealed an average blood loss of three ml. per day during salicylate administration. The amount of blood which the subjects lost by venipuncture for the necessary determinations was calculated as 45 ml. per week. Measurements during an observation period of two weeks on two normal subjects who were bled, up to a total of 135 ml. each, revealed no significant changes in serum iron during this period. When the same two volunteers were then given salicylates for seven days, a prompt drop in serum iron oc-

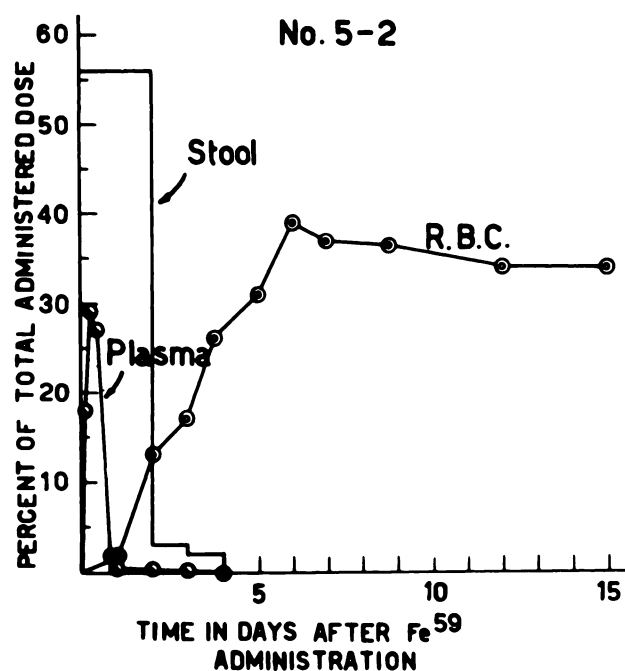


Fig. 5.—Case No. 5. For legend, see figure 4. Test was carried out during the administration of salicylates; absorption is normal, utilization increased.

curred. These results seem to indicate that blood loss was not the cause of the drop in serum iron in our subjects.

It was also shown in the tracer studies that no impairment of iron absorption occurred. Nor was there an increased excretion of iron in the form of a complex in the urine during the administration of salicylates. Therefore, the precipitous drop in the serum iron values cannot be explained by direct loss of iron in the urine.

The cause of the drop in serum iron values in patients receiving salicylates remains, therefore, unclear. It is conceivable that the salicylates alter the function of the reticuloendothelial system, as a result of which circulating plasma iron is removed from the circulation and deposited in the reticuloendothelial cells. Further work is going on in this laboratory to test the above hypothesis.

In addition to a decrease in serum iron, most of the patients in whom these studies were carried out showed a decrease in hemoglobin and hematocrit. While the amount of blood lost through the gastrointestinal tract was not sufficient to explain the hypoferrremia and anemia observed, the possibility must be considered that the bleeding may have contributed to the decrease in circulating hemoglobin in these patients during salicylate administration. The combination of blood loss and increased red cell destruction may account for the anemia which developed following salicylate administration.

These observations form an additional indication that the prescription of even a common and innocuous compound may have more profound effects on the body economy than is usually realized. Whether smaller dosages of

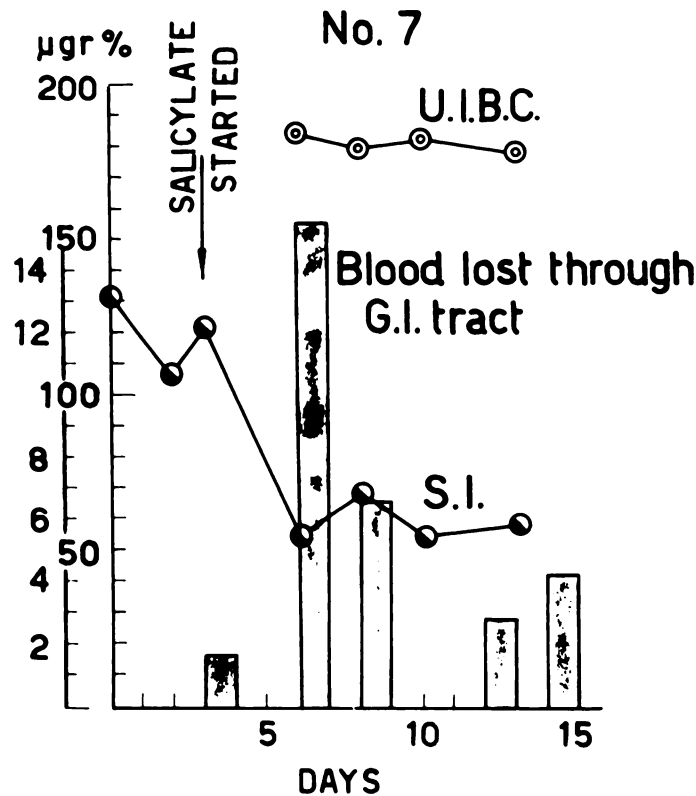


Fig. 6.—Case No. 7. Illustrating the drop in serum iron and the amount of blood lost in the stools (by Cr^{51} method) during administration of salicylates.

salicylates than those used by our patients have similar effects and whether the wide-spread use of salicylates is a factor of importance in the production of anemia, especially in patients with rheumatic manifestations, remains to be investigated.

SUMMARY

In nine patients with various diseases and in two normal subjects, administration of acetylsalicylic acid (aspirin) in amounts of 3–4 Gm. per day produced a drop in serum iron. This drop could not be explained by chemical interference with the determination, by blood loss, impaired absorption or increased excretion of iron. In addition, it was shown that during administration of salicylates the survival time of the red cells was diminished. It is suggested that salicylates might produce anemia not only by gastrointestinal bleeding but also by interference with the metabolism of iron through some unknown mechanism.

SUMMARIO IN INTERLINGUA

In novem patientes con varie morbos e in duo subjectos normal, le administration de acido acetylsalicylic (aspirina) in doses de 3 a 4 g per die produceva un declino in le contento de ferro in le sero. Iste declino non poteva

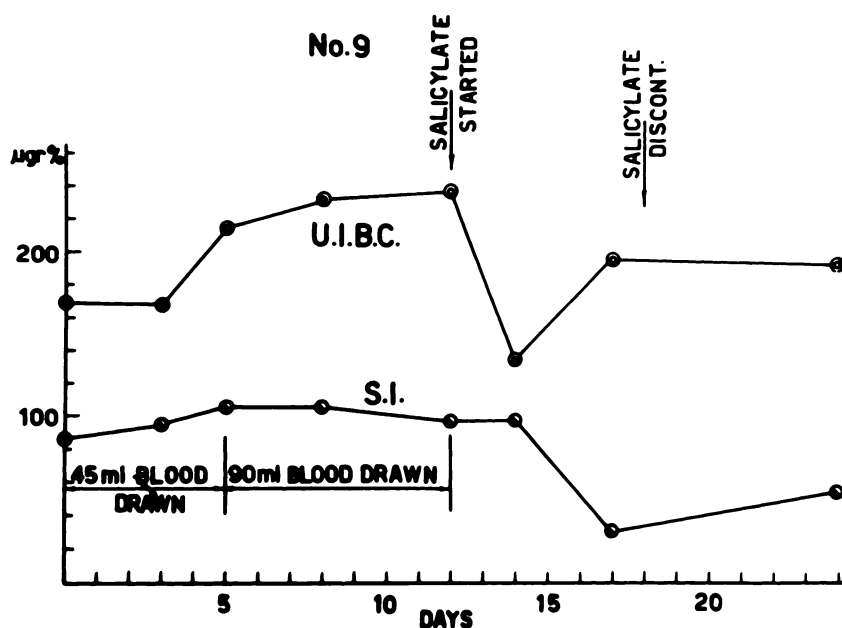


Fig. 7.—Normal Subject No. 9. Repeated bleedings, totaling 135 ml. during 12 days have no effect on serum iron; salicylate administration produces a prompt drop within one week.

esser explicate per factores chimic interferente in le determination ni per perditas de sanguine ni per un defecto del absorption o un aumento del excretion de ferro. In plus, il esseva demonstrate que durante le administration de salicylato, le tempore de superviventia del erythrocytos esseva reduceite. Es proponite le these que salicylato produce anemia non solo per sanguination gastrointestinal sed etiam per un interferentia in le metabolismo de ferro del parte de un mecanismo non ancora identificate.

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