STUDIES OF LIVER FUNCTION IN SUBJECTS RECEIVING METHOXSALEN*

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The chemical configuration of methoxsalen has been somewhat disturbing to investigators interested in hepatic function because of possible hepatotoxic activity. Some meager preliminary data suggesting that selective damage might occur was based on the finding of questionable elevation of occasional cephalin-cholesterol flocculation tests. A systematic study of liver function with methoxsalen seemed indicated particularly in view of the projected widespread clinical trial which has since taken place over the last several months. Elliott in 1956, reporting on 22 patients receiving the drug, stated that two developed elevated bromsulphalein retention. Pretreatment examinations of liver function were not available so that the precise significance of these elevated tests is uncertain.

Since our own concern with the problem stemmed from an interest in metabolism with particular emphasis on liver function, we focused the resources of our laboratory on the possibility that methoxsalen might be hepatotoxic. In designing this experiment the following tests of liver function were selected since they each have peculiar advantages.

First, the bromsulphalein test was selected because it is one of our more sensitive indicators of liver dysfunction in the absence of jaundice, picking up early disturbances in function; it is also reproducible and easily standardized. It is fairly specific although there is some evidence that peripheral uptake of the dye does occur. Over a period of years, however, we have by now been witness to more than ten thousand bromsulphalein examinations so that our confidence in the clinical interpretation of this test is firmly based.

The thymol and zinc turbidity tests, while non-specific for liver function, are found to be most often disturbed in the presence of liver disease or injury. These turbidity tests are simple measurements of the cloudiness resulting when saturated solutions of either zinc sulfate or thymol are added to approximately 0.1 cc. of serum. The resulting turbidity depends on the presence of the precipitating agent (zinc or thymol) and some lipoprotein which changes in concentration in the serum during periods of liver damage. In addition, there are changes in gamma and beta globulin that reflect alterations in liver function, the zinc test being particularly associated with gamma globulin changes.

The cephalin-cholesterol flocculation test is a sensitive screening test and was added to the experiment because it reflects the diffuseness of the hepatic lesion. In addition, it should be emphasized that the tests singly mean very little, but when all are done simultaneously the resulting information is greatly enhanced in value. Our laboratory is geared almost completely for the study of liver function so that we have the advantage of a long period of standardization of procedures as well as long experienced technical personnel.

In the present study 25 medical students were selected and divided into two groups of 12 each. One student was discarded because preliminary testing revealed a 4+ cephalin flocculation test. The 12 test subjects were randomly selected and received daily one dose of 30 mg. of methoxsalen, 12 receiving the drug, and the 12 others received placebos. The subjects and the physicians were unaware of which drug was being used. (Dr. Fowlks of our group detected fluorescence in the sera of those patients receiving the drug, but he did not know their names nor did those of us involved know that Dr. Fowlks' serum tests were revealing those subjects on drug or placebo at the time the experiment was in progress.) All volunteers were in good state of nutrition and were basically in good health.

Table 1 shows the results of the pre- and post-treatment period on the serum cephalin-cholesterol flocculation tests. At the end of twenty-four hours, the serum showed that eight of the 12 had no flocculation, four had a 1+ reaction, etc. At the end of 48 hours, 7 of the 12 were still zero, and 2 had a 1+. The placebo group was no differ-

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Presented at the Brook Lodge Invitational Symposium on the Psoralens, sponsored by The Upjohn Company, Kalamazoo, Michigan, March 27–28, 1958.

TABLE 1

Results of cephalin-cholesterol flocculation tests performed at monthly intervals to compare 12 subjects taking placebos with 12 subjects taking 30 mg. methoxsalen per day

Cephalin-Cholesterol Flocculation

12 8-MOP Subjects and 12 Placebo Subjects

	Control		1 Month		2 Months		3 Months	
	8-MOP	Placebo	8-MOP	Placebo	8-MOP	Placebo	8-MOP	Placebo
0 24 Hr.	8	7	10	9	12	9	7	6
0 48 Hr.	7	5	10	8	11	9	1	1
1+ 24 Hr.	4	4	0	2	0	1	5	6
1+ 48 Hr.	3	2	0	2	1	1	11	10
2+ 24 Hr.	0	1	2	1	0	1	0	0
2+ 48 Hr.	2	5	2	2	0	1	0	1
3+ 24 Hr.	0	0	0	0	0	0	0	0
3+ 48 Hr.	0	0	0	0	0	0	0	0

ent significantly since at the end of 24 hours 7 were zero, and at the end of 48 hours 5 had a zero, etc. One month after starting therapy, 10 had a zero, and at the end of 48 hours 10 were still zero. At the end of 48 hours a 1+ appeared. There were 2+ reactions in the drug group, and two 2+ reactions also in the placebo group. At two months the findings were not much different, and at three months had changed not at all. I might say that the normal limits in our laboratory go to 2+, and one can see that there were no reactions beyond this either in the placebo or drug-treated group.

The thymol turbidity reactions have been shown in Figure 1. Our normal limits are six units or less, and during the control period one can see that the ranges, according to the black lines crossing the baseline, fall well within the normal range. The open circles represent placebo controls. While there is some variation in the mean, it stays well within the normal range, but over a period of three months and, indeed, at the end of three months of therapy, the entire range is well within the normal zone and the variation is extremely small. The occasional instance of a 4.2 unit reading is still acceptably normal. Our

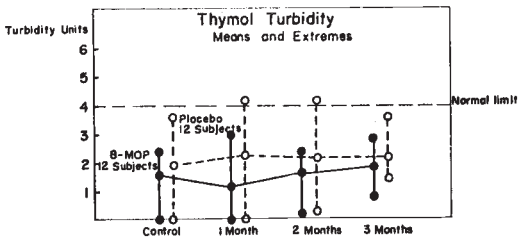

FIG. 1. Means and extremes of thymol turbidity reactions done at monthly intervals to compare 12 subjects taking placebos with 12 subjects taking 30 mg. methoxsalen per day.

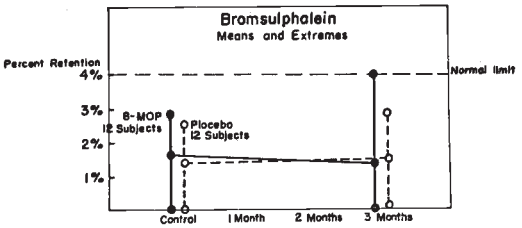

FIG. 2. Means and extremes of BSP retentions performed on 12 subjects before and after ingestion of 30 mg. methoxsalen per day for three months compared with BSP retention of 12 subjects taking placebos.

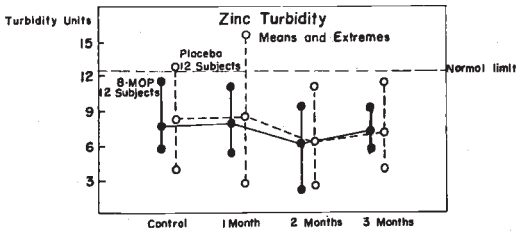

FIG. 3. Means and extremes of zinc turbidity reactions performed at monthly intervals to compare 12 subjects taking placebos with 12 subjects taking 30 mg. methoxsalen per day.

laboratory would not feel that tests of this order of magnitude would be significant since they are unsupported by aberrations in any of the other tests. Clinical examinations done on these subjects throughout the period of testing showed no abnormalities.

The bromsulphalein examinations, summarized in Figure 2, also show no abnormalities, and the mean values fall within the middle zone of acceptable normal.

The zinc turbidity reactions (Figure 3) showed little abnormality except for one single elevation beyond accepted normal on one instance, but, again, this was not reinforced by abnormalities in the other tests.

Although 75 or 80 liver function tests are

known, and any or all of them might have been applied to this experiment, it seemed feasible for practical considerations to select these few for their unique characteristics and apply them in combination. This reinforced the strength of our experimental design considerably.

Clinically, there were only occasional periods of epigastric distress. One patient complained of nasal distress, one vertigo, but otherwise no extraordinary signs or symptoms developed. I think it is also important to note that one group received therapy in the early part of our winter from October through December, and another from December through February. This is the period in Portland, Oregon, for the peak incidence of hepatitis. Were there even slight amounts of hepatic damage from the drug, creating possible increased vulnerability to hepatitis, these were not shown by the laboratory or clinical examinations. Being medical students, of course, they were exposed around the hospitals and clinics to patients with diseases of all varieties. Secondly, it is also of interest that this period of experimentation coincided with the period of mid-term and final semester examinations when bouts of moderate alcoholism among medical students are not unknown! All I can say is that in spite of two possible stress experiences, there was no evidence of liver dysfunction, and all tests stayed well within normal limits.

These data then show that none of the 12 subjects receiving methoxsalen developed changes in hepatic function. It is possible to state with 95% confidence only that no more than 27% of a population like this group of subjects may develop a change in liver function. These figures have been evolved by the work of our biostatisticians, especially Dr. Carl Hopkins and Dr. Farrington Daniels, Jr., who have been interested in the confidence values for an experiment involving only 24 men. In other words, as they have expressed it, anywhere from 100% to 73% of individuals ingesting 30 mg. of methoxsalen daily for three months will not have a change in hepatic function as detected by the tests we have used in this study. The small sample size limits the sensitivity of the experiment, and it will not allow a more positive statement. Our consultants tell us that to be more certain of a negative effect of a drug for 99% of the population would require an experimental group of at least 450 subjects.

While I would not diminish the firm convictions that arise from such statistical confidences, I would like to think that our considerable practical experience in the interpretation of liver tests in the field of hepatic injury carries with it a more favorable confidence factor—one that probably does not lend itself easily to statistical expressions.