membrane filter in a sterilized filtration apparatus. Membranes were mounted in petri plates that contained a few milliliters of the following media: (i) sterilized river water, (ii) 1.5 percent agar, (iii) 0.1 percent peptone, and (iv) 0.1 percent dextrose. These were incubated in inverted position for 48 hours at room temperature, prior to counting under a dissecting microscope. The average density of 15 squares was used to estimate the total number of colonies on the filter.

Relative photosynthetic rates were measured with ^{14}C; we followed, in general, the light and dark bottle procedure described by Strickland and Parsons (*2*) for measurement of productivity by means of the ^{14}C method. Twelve bottles were used in each experiment. One set of three (two light and one dark) filled with intake water was incubated at ambient water temperature, another set of three at the temperature of the effluent. Similar sets of bottles filled with effluent water were incubated at ambient and at effluent temperature.

The wood and plexiglass incubators were filled with tap water circulated through a reservoir fitted with a cooling unit and a heating unit to maintain the desired temperature. Temperatures were maintained within 2°C of that designated during the course of incubation. Although the absolute temperature of the incubators was different on the several experimental days, the differential was nearly the same (Table 1). Each incubator contained eight 40-watt fluorescent lamps. Average light intensities were 0.89×10^4 erg cm^2 sec^{-1} and 1.12×10^4 erg cm^2 sec^{-1} (*3*). No correction has been made in the data for the slight difference in light value.

Following a 3- to 4-hour period of incubation, experiments were concluded by filtration of 25 or 50 ml from each bottle through a 0.45-μm membrane. These were mounted on ring and disk assemblies, desiccated, and counted in a thin end-window, gas-flow counter (Nuclear-Chicago).

A conspicuous reduction of photosynthetic rate is apparent in experiments 1–4 (Table 1). As the plant was chlorinating at these times, we believe this is a direct effect of chlorine in the effluent sample. The reductions are statistically significant and are paralleled by reductions in bacterial densities and concentrations of chlorophyll a. A similar though smaller reduction in photosynthesis occurred in experiment 5. Although this is not statistically significant, it is paralleled by a lower bacterial count and chlorophyll concentration. We are unable to explain this result; no application of chlorine was recorded at this time. The percentage reduction in photosynthesis (ignoring the difference in incubation temperatures) was calculated as follows:

$$\% \text{ reduction} = \left(1 - \frac{\text{mean of effluent rates}}{\text{mean of intake rates}}\right) 100$$

Rates of photosynthesis, concentrations of chlorophyll a, and bacterial counts are nearly identical in experiments 6–11, although some of the differences in rates of photosynthesis are significant.

Most of these differences may be interpreted as being due to temperature increase. In the last six experiments (as well as four of the initial five) heated intake water has a higher rate of photosynthesis than does intake water incubated at ambient temperature. The same is true of effluent water in experiments 5–11. Any effect temperature might have had in effluent water in experiments 1–4 is evidently overshadowed by the effect of chlorine.

Our results are similar to those of Warinner and Brehmer (*4*) in the York River. Comparability is limited, however, since they employed generally larger temperature differentials. Also, the differentials were induced in the laboratory, rather than by plant operations, which thus excluded any influence of chlorine. Morgan and Stross (*5*) also found reductions and increases in primary productivity in work more comparable to our own at the same site.

The maximum rate of water use by this plant has been estimated to be 30 percent of the rate of supply of new water to the adjacent segment of the river (*6*). Chlorination records (*7*) show that only rarely is chlorine applied more than 6 hour day^{-1} at Chalk Point; application 25 percent of the time would thus be a reasonable maximum. Ignoring intensity and timing of application, and assuming 91 percent loss of production when chlorination occurs, a maximum loss of primary production of 6.6 percent may be calculated for the affected area of the river. Whatever the exact magnitude of this loss in the condenser water, we have not detected a consistent reduction of primary production in the vicinity of the outfall of the effluent canal in field studies.

D. H. Hamilton, Jr., D. A. Flemer
Carolyn W. Keefe, J. A. Mihursky
Chesapeake Biological Laboratory, University of Maryland Natural Resources Institute, Solomons 20688

References and Notes

1. C. S. Yentsch and D. W. Menzel, *Deep-Sea Res.* **10**, 221 (1963).
2. J. D. H. Strickland and T. R. Parsons, *Bull. Fish. Res. Board Can.* **167** (1968).
3. Light intensities were measured in situ in the incubator with a Yellow Springs Instrument radiometer.
4. J. E. Warinner and M. L. Brehmer, *Int. J. Air Water Poll.* **10**, 277 (1966).
5. R. P. Morgan and R. G. Stross, *Chesapeake Sci.* **10 (3-4)**, 165 (1969).
6. D. W. Pritchard, *Nuclear Power Plants in Maryland, a Report by the Governor's Task Force on Nuclear Power Plants* (State Office Building, Annapolis, Maryland, 1969), Append. D, 108 pp.
7. The 6 hour day^{-1} estimate is based on examination of monthly records covering daily operation for a 2-year period. These records are available at the Department of Water Resources, State Office Building, Annapolis, Md.
8. Supported by contract No. 14-01-0001-1969 with the Office of Water Resources Research, U.S. Department of the Interior. Contribution No. 420, Natural Resources Institute of the University of Maryland. We thank D. R. Heinle (Chesapeake Biological Laboratory) and J. Carpenter (Chesapeake Bay Institute) for comments and suggestions and R. C. Whaley (Chesapeake Bay Institute) for constructing the incubators.

10 March 1970; revised 22 May 1970

Acetylsalicylic Acid: No Chromosome Damage in Human Leukocytes

Abstract. *Acetylsalicylic acid was added to cultures of human leukocytes at several time periods over a wide range of concentrations (0.1 to 300.0 micrograms per milliliter). Leukocytes were also cultured from human volunteers during the ingestion of two 300-milligram tablets four times daily (2400 milligrams per day) over a 1-month period. No significant increase in chromosome aberrations was detected in vitro or in vivo.*

The potential for carcinogenesis and long-term genetic damage in man from environmental agents (radiation, viruses, chemicals, drugs, food additives, and other substances in common usage) is a subject of widespread concern. One means of assessing this potential is through short-term cytogenetic tests, with primary reliance placed on the effect of such agents on the chromosomes and growth of peripheral leukocytes in vitro. We have

initiated such a preliminary screen for studies of chemical mutagenesis with acetylsalicylic acid (ASA; aspirin). Recent reports have indicated this widely used analgesic caused chromosome damage in vitro at concentrations "far lower than the blood-level of 100–120 μg per ml 2 hours after the ingestion of 2.0 g" (*1*). Specifically, in the only cytogenetic investigation which, to the best of our knowledge, exists in the world literature, Jarvik and Kato (*1*) reported an average breakage frequency twice the control value (10.0 percent versus 4.7 percent) in normal human leukocyte cultures treated with 0.1 and 1.0 μg of ASA per milliliter of medium 4 hours before harvesting of cells (that is, from 68 to 72 hours of culture). If aspirin is accepted as a chromosome-breaker, one of two conclusions is unavoidable: either (i) the clinical manifestations of this damage are incredibly insidious or (ii) the test system utilized is far too delicate or inconsequential to be of any value. In order to resolve either of the above conclusions, more extensive studies of ASA were deemed essential. We have been unable to confirm these results (*1*) in over 80 leukocyte cultures to which ASA was added, over several exposure times, to the limits of its solubility in aqueous media (about 300 μg/ml); nor does ASA apparently damage leukocyte chromosomes in vivo at plasma concentrations well above the levels used by Jarvik and Kato in their in vitro studies (*1*).

The microculture method for culturing leukocytes (*2*), with minor variations, was used for cultures to which ASA was to be added. Blood flowing from a finger prick was drawn into a 1.0-ml tuberculin syringe which had been fitted with a 26-gauge needle and prerinsed with sodium heparin; after removal of needle, two drops (approximately 0.1 ml) were added to tubes, each of which contained 5 ml of culture medium (*3*). Acetylsalicylic acid, made up as a sterile stock solution in culture medium (*4*), was added to cultures at different times: at the initiation of culture, or at 48 hours, or at 68 hours. Replicate cultures were run for each concentration of ASA used in vitro and for each time period of exposure. Control cultures received an equivalent volume of diluent [distilled water or Eagle basal (BME) culture medium]. The cultures from volunteers (*5*) on a regimen of two ASA tablets (*6*) four times daily for 1 month were established by a modifica-

Table 1. Chromosome breakage frequency in human leukocytes exposed in vitro to acetylsalicylic acid (ASA). T_0, ASA added at initiation of culture; T_{48}, ASA added 24 hours before harvest; and T_{68}, ASA added 4 hours before harvest.

ASA conc. (μg/ml)	Time of addition	Donor M.L.				Donor L.K.				Donor D.W.				Donor I.M.			
		Percentage of mitosis*	No. of metaphases	Percentage of breaks	Limits†	Percentage of mitosis*	No. of metaphases	Percentage of breaks	Limits†	Percentage of mitosis*	No. of metaphases	Percentage of breaks	Limits†	Percentage of mitosis*	No. of metaphases	Percentage of breaks	Limits†
0		100	397	0.8	0.16– 2.0	100	392	0.3	0.01–1.4	100	253	1.2	0.2– 3.5	100	411	1.0	0.3 – 2.5
0.1	T_{68}	82	150	2.0	.4 – 5.3	122	93	2.1	.3 –7.3					102	100	1.0	.03– 5.6
1.0	T_{68}	91	100	2.0	.3 – 6.8	119	100	1.0	.03–5.0					97	100	3.0	.6 – 8.8
	T_0	109	82	6.1	2.0 –13.3	88				91	104	<0.9	0 – 3.6	107	107	1.9	.2 – 6.7
12.5																	
	T_{48}	95	104	<0.9	0 – 3.5	112	100	1.0	.03–5.0					92	101	<1.0	0 – 3.7
	T_0	112	108	3.7	1.0 – 9.5	90				85	213	1.4	0.3– 4.1	68	104	3.8	1.0 – 9.8
25.0																	
	T_{48}	92	104	0.9	.02– 5.4	126	103	1.0	.03–5.0	90				62	101	<1.0	0 – 3.7
	T_0	90	150	2.0	.4 – 5.8	90				91	190	1.6	.3– 4.6	77	106	0.9	0.02– 5.3
50.0																	
	T_{48}	103	104	<0.9	0 – 3.5	164	103	<1.0	0 –3.6					64	55	<1.8	0 – 6.7
	T_0									51	56	<1.8	0 – 6.6				
125.0																	
	T_{48}	73	50	<2.0	0 – 7.4	102	50	<2.0	0 –7.3	98	50	<2.0	0 – 7.4	92	51	<2.0	0 – 7.2
250.0	T_{48}	75	51	2.0	0.1 –10.9	66	132	<0.8	0 –2.8	85	50	<2.0	0 – 7.4	75	53	4.0	0.5 –12.8
300.0	T_{48}	65	50	4.0	.5 –13.5	77	50	2.0	0.05–9.9	47	50	4.0	0.5–13.5	59	50	2.0	.05– 9.9

* As compared to controls. † 95 percent confidence limits determined from tables of Stevens (*13*).

Table 2. Incidence of chromosome breakage in cultured leukocytes from human volunteers during program of two aspirin tablets (600 mg) four times daily for 1 month. The highest individual breakage frequencies were not related to the concentration of salicylate; in no individual case was chromosome breakage different from the control (pretreatment) value during the entire treatment period. Pre, before administration of ASA; 24 hours, and so forth, time after start of ASA administration.

Time blood sample obtained	No. of volunteers	Mitotic indices*	Salicylate conc. in plasma (μg/ml)	No. of metaphases observed	Percentage of breaks
Pre	5	43–66		271	2.0
24 hours	6	31–67	33–94	481	2.0
72 hours	4	29–53	47–135	125	<0.8
2nd week	7	44–86	45–139	538	1.7
3rd week	5	33–60	28–119	300	1.7
4th week	3	36–57	32–123	200	0.5

* Number of dividing cells per 1000 cells counted.

tion of the standard macromethod (*7*). Approximately 5.0 ml of blood were removed by venipuncture into heparinized Vacutainers, the erythrocytes were allowed to settle by gravity sedimentation over a period of 2 to 3 hours at room temperature, and all of the leukocyte-rich plasma from each sample was equally distributed to two culture tubes containing 5.0 ml of GIBCO (Grand Island Biological Co.) Medium 1A (approximately 0.5 ml of plasma per tube). A blood sample was drawn on two occasions prior to the first dose of aspirin and at 24, 48, and 72 hours after the first dose; thereafter, specimens were taken at weekly intervals. Concentrations of salicylate in plasma were determined by the procedure of Trinder (*8*). All cultures from both studies were incubated for 72 hours at 37°C. Preparation of metaphase spreads followed the standard procedures for leukocyte cultures (*9*), including a 2.5-hour exposure to Colcemid, hypotonic treatment with calf serum diluted 1:5 with distilled water, fixation in a mixture of methanol and acetic acid, blaze-drying (*10*), and staining with Giemsa. Chromosome aberrations were scored according to the criteria summarized by several workers (*11*).

Table 1 summarizes the findings after exposures of leukocyte cultures to 0.1 to 300 μg of ASA per milliliter at various time periods. The lower concentrations (0.1 and 1.0 μg/ml), added 4 hours before harvesting (T_{68}), represent an attempt to repeat the work of Jarvik and Kato. All other experiments were designed for exposures of 72 hours (T_0) (that is, for the entire incubation period) or for 24 hours (T_{48}), in order to uncover both simple chromosome breakage as well as any exchange figures (dicentrics, tri- and quadriradials, and so forth), the latter resulting from multiple breakage and fusion of chromosomes in daughters of surviving cells undergoing more than one division (*12*). No significant elevation of simple chromosome aberrations (breakage) was observed at any of the concentrations of ASA tested, nor were any complex chromosomal rearrangements seen in any culture. The percentage of polyploid cells (mainly tetraploids) was similar in both experimental and control cultures (< 1.0 percent). Severe growth inhibition occurred in cultures treated throughout the 72-hour incubation period with 125 μg/ml and higher concentrations; consequently, with the exception of one culture, exposures to the higher doses were restricted to the last 24 hours of culture (Table 1). A test for more subtle statistically significant differences was applied according to the method of Stevens (*13*). No differences were revealed. By contrast, in preliminary studies with caffeine, consistent dose-related chromosome damage was induced in cultures set up from the same individuals according to the same protocols at concentrations of 250 through 2000 μg/ml.

Table 2 gives the results from individuals who had been taking 2.4 g of aspirin per day (in divided doses) for 1 month. No increased chromosome breakage (or other aberration) or growth inhibition was observed. Despite the relatively wide range of salicylate levels (marked variations were recorded both between individual subjects and between sequential samples from each individual), all were above the levels used by Jarvik and Kato (*1*).

The lack of significant in vitro chromosome damage by the extensively used analgesic acetylsalicylic acid over the wide range of concentrations used in our study indicated that this drug cannot be considered dangerous by the leukocyte test system, nor can this test be dismissed as being too delicate to be of any consequence. In vivo cytogenetic testing of ASA has not heretofore been reported. However, the comparable lack of damage to chromosomes, when the same cell test system (peripheral leukocytes) is used, does not preclude chromosome aberration by ASA in other human tissues not yet sampled, nor its potential for mutagenesis, for example, in germ cells. Another commonly used substance, caffeine, has been estimated to be present in human gonadal and fetal tissue at levels of 1 μg per milliliter of tissue water, the genetic hazard of such chronic exposure being at present unknown but still a matter of intense interest (*14*).

Further investigations of acetylsalicylic acid are warranted to remedy the paucity of information on its chromosome-damaging and mutagenic potential, as well as its metabolism and pharmacological action. The question of its teratogenicity in man remains unanswered; in animal studies, massive doses of ASA (toxic equivalents in man) fed to rats and mice have caused fetal death and resorption, as well as cleft lip and other malformations in two inbred strains of mice (*15*).

Note added in proof: Recently, Meisner and associates (*16*) reported no dose-related chromatid breakage after the addition of ASA to continuous cultures of human skin; 1 week after two treatments with 250 μg/ml, a total of 2.3 percent chromosome aberrations was recorded (the average control value was 1.7 percent).

IRVING MAUER
DAVID WEINSTEIN
Cytogenetics Group, Department of Experimental Pathology and Toxicology, Hoffman-La Roche Inc., Nutley, New Jersey 07110
HARVEY M. SOLOMON
Hoffmann-La Roche Special Treatment Unit, Martland Hospital, Newark, New Jersey 07110

References and Notes

1. L. F. Jarvik and T. Kato, *Lancet* **1968-I**, 250 (1968); L. F. Jarvik, *Science* **162**, 621 (1968); T. Kato and L. F. Jarvik, *Dis. Nerv. Syst.* **30**, 42 (1969).
2. J. H. Edwards, *Cytogenetics* **1**, 90 (1962); D. T. Arakaki and R. S. Sparkes, *ibid.* **2**, 57 (1963).
3. Either GIBCO's Chromosome Medium 1A, available completely prepared in liquid form and containing all the components necessary for leukocyte culture, or Flow's AutoPow BME, an incomplete medium, to which the following additions were made for optimum cell growth: 15 percent fetal calf serum, 1

percent each of BME amino acid and vitamin solutions (100×, from GIBCO), and 2 percent phytohemagglutinin (Burroughs Wellcome).
4. Dow Chemical ASA, lot No. 36-023-01, dissolved at 37°C in GIBCO-BME culture medium.
5. Nine healthy adults ranging in age from 21 to 48 years participated in the study. X-ray technicians, subjects who have had diagnostic x-ray procedures other than chest x-rays, subjects exposed to x-rays during the previous 6 months, individuals with upper respiratory tract infections or other recent viral diseases, individuals taking any medications chronically, pregnant females, subjects with a history of peptic ulcer or bleeding diathesis, and individuals allergic to aspirin were excluded from the study.
6. Bayer Aspirin, 300-mg tablets, lot No. D 8078.
7. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Batipps, D. A. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
8. P. Trinder, *Biochem. J.* **57**, 301 (1954).
9. W. J. Mellman, in *Human Chromosome Methodology*, J. J. Yunis, Ed. (Academic Press, New York, 1965), p. 21.
10. R. G. Scherz, *Stain Tech.* **37**, 386 (1962).
11. T. H. Chang, P. S. Moorhead, J. G. Boué, S. A. Plotkin, J. M. Hoskins, *Proc. Soc. Exp. Biol. Med.* **122**, 236 (1966); M. M. Cohen, K. Hirschhorn, W. A. Frosch, *N. Eng. J. Med.* **277**, 1043 (1967).
12. Using tritiated thymidine pulse labeling, M. A. Bender and D. M. Prescott [*Exp. Cell Res.* **27**, 221 (1962)] have suggested that leukocytes may undergo as many as four divisions in culture.
13. W. L. Stevens, *J. Genet.* **43**, 301 (1942).
14. A. Goldstein and R. Warren, *Biochem. Pharmacol.* **11**, 166 (1962).
15. D. G. Trasler, *Lancet* **1965-I**, 606 (1965); H. J. Klein Obbink and L. M. Dalderup, *ibid.* **1964-I**, 565 (1964); ———, *ibid.* **1964-II**, 152 (1964).
16. L. F. Meisner, S. L. Inhorn, P. M. Nielson, paper presented at Somatic Cell Genetics, 8th Conference, Chatham, Mass., 7–10 October 1969.
17. We thank Marion L. Katz and Lanny Katz (Cytogenetics Group) for technical assistance; Marian Barakat and Connie Ashley (Special Treatment Unit) for collecting blood samples from volunteers; and the subjects who participated in the in vivo study.

17 February 1970; revised 6 April 1970

In vivo Conversion of ^{3}H-L-Tryptophan into ^{3}H-Serotonin in Brain Areas of Adrenalectomized Rats

Abstract. *Rats were adrenalectomized 10 days before we estimated in vivo the conversion index of ^{3}H-tryptophan into radioactive serotonin in brainstem and telediencephalon. We found that the conversion index in the brainstem of adrenalectomized rats is smaller than in the same area of sham-operated rats. Conversely, the conversion index in the telediencephalon was similar in the two groups of rats. The serotonin concentrations were unchanged by adrenalectomy, which suggests that in brainstem the decrease of tryptophan hydroxylase is reflected by the conversion index estimation and not by measurement of serotonin steady-state concentrations.*

Studies on the localization of serotonin (*1*) in brainstem have revealed that most of the serotonin and perhaps the tryptophan hydroxylase activity is associated with cell bodies, where the enzyme is probably synthesized. Corticosterone, the main component of the secretion of rat adrenal glands (*2*), stimulated de novo synthesis of tryptophan hydroxylase in the midbrain when injected into bilaterally adrenalectomized rats (*3*). In these animals the activity of the midbrain tryptophan hydroxylase was reduced by 75 percent when compared with that of sham-operated rats (*3*). Since tryptophan hydroxylase is the rate-limiting enzyme for serotonin biosynthesis (*4*), one might expect that adrenalectomy or injections of adrenal corticosteroids should change the concentrations of brain serotonin in opposite directions. This prediction is not supported by reports which show that adrenalectomy can decrease (*5*), increase (*6*), or leave unchanged (*7*) whole brain serotonin content, while cortisone injections can either increase (*8*) or fail to change (*9*) the brain concentrations of serotonin.

A discrepancy between turnover rate of serotonin and the steady-state concentrations of brain serotonin was reported following inhalation of 100 percent oxygen (*10*) during ether anesthesia (*10*) and after LSD injections (*11*). Therefore, we decided to test whether the reported reduction of tryptophan hydroxylase in the rat midbrain after bilateral adrenalectomy (*3*) changed the in vivo conversion rate of tryptophan (TP) into midbrain serotonin and decreased the serotonin concentrations in midbrain. We have compared the conversion indexes of TP into serotonin in brainstem and in telediencephalon of sham-operated and adrenalectomized rats receiving a pulse injection of ^{3}H-TP. This conversion index (CI) was calculated as proposed by Sedvall *et al.* (*12*) from the relationship

$$CI = \frac{\text{nc serotonin/g}}{\text{nc TP/nmole TP}} \quad (1)$$

where the conversion index has the dimension of nanomoles of serotonin. This formula neither corrects for the continuous efflux rate of the ^{3}H-serotonin from brain nor for the rapid changes of the TP specific activity at the initial times after labeling (*13*). Although the conversion index is not a measure of serotonin biosynthesis, a comparison of the conversion index in different experimental situations may be used to approximate whether or not the turnover rate of serotonin has changed.

Male, Sprague-Dawley rats (180 to 220 g) were bilaterally adrenalectomized or sham-operated by Zivic Miller, Pittsburgh, Pennsylvania. The animals were housed in large group cages upon delivery and maintained with free access to a NaCl solution (0.9 percent) and standard lab chow. A pulse intravenous injection of 500 μc of ^{3}H-L-tryptophan per kilogram (5 c/mmole, New England Nuclear Co.) in 10 ml of phosphate buffer per kilogram (7.4 *p*H, 0.2*M*) was made in the afternoon of the tenth postoperative day and the rats were decapitated either 20 or 40 minutes later. Sham-operated and adrenalectomized rats used as controls received only phosphate buffer. The brain was quickly removed and dissected into brainstem (pons, medulla, and midbrain) and telediencephalon. The latter included all structures anterior to a cut from the anterior part of the superior colliculus to the posterior edge of the mammillary bodies (*14*). These two brain areas were immediately frozen and they were kept frozen until assay of the specific activity of TP and serotonin (*15*). Blood was collected in heparinized tubes and immediately centrifuged to separate the plasma where we assayed TP (*15*) and corticosterone concentrations (*16*). Radioactive TP and serotonin were estimated by liquid scintillation spectrometry in 2.0-ml aliquots from the aqueous phases used to estimate fluorimetrically the amine and the amino acid concentrations.

The average concentration of corticosterone in plasma of sham-operated rats was 34.2 μg/100 ml and that of adrenalectomized rats was 2.7 μg/100 ml. Adrenalectomized rats with concentrations of plasma corticosterone greater than 10 μg/100 ml were discarded.

Table 1 lists the concentrations and specific activity of TP in plasma, brainstem, and telediencephalon of adrenalectomized and sham-operated rats 20 and 40 minutes after the injection of ^{3}H-TP. The concentration of TP of adrenalectomized rats was significantly greater than that of sham-operated rats only in the plasma of experiment 2 (Table 1).

Following adrenalectomy the decrease