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Effect of *Corynebacterium parvum* on Liver Proliferation and Regeneration[1]

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ABSTRACT

The observation that *Corynebacterium parvum* (CP) administration results in hepatomegaly prompted us to investigate the effect of that agent on hepatocyte proliferation in normal and partially hepatectomized rat livers. Its i.v. administration to normal rats with intact livers resulted in a significant increase in liver DNA synthesis (tritiated thymidine uptake), total liver DNA, and liver weight, which were maximal at 4, 7, and 9 days, respectively. Both weight and DNA content remained elevated for at least 56 days after CP injection. Autoradiography carried out at the time of maximal DNA synthesis indicated that hepatocyte proliferation contributed substantially to the increased tritiated thymidine uptake. Histological examination of livers from treated animals revealed an increased number of Kupffer cells, macrophage granuloma formation, and focal areas of hepatocyte necrosis. When smaller doses of CP, which resulted in less extensive histological changes, were used, increases in liver DNA synthesis, DNA content, and weight were similar to those occurring after use of a larger dose. The effect of CP was compared with that obtained when other immunomodulators were used. If a second injection of CP was administered at the time when DNA synthesis was maximal or when it had nearly returned to control values, further stimulation of DNA synthesis failed to occur.

When CP was given 1 day before or after partial hepatectomy, liver regeneration was greater than it was in untreated hepatectomized controls. Liver remnants at the time of partial hepatectomy were larger when CP had been administered 7 days previously than were those in untreated controls. Consequently, although liver weights at time of sacrifice were similar in the two groups, the percentage of regeneration was less in CP-treated animals. Such a finding further indicates that the hepatomegaly following CP is related to increased numbers of hepatocytes since liver regeneration is inversely related to the liver remnant following partial hepatectomy. While liver damage resulting from CP administration may be responsible for the liver regeneration observed, other factors have been considered.

INTRODUCTION

The use of CP[3] in experimental animal systems has been demonstrated to produce a variety of changes in liver. Hepatomegaly has been a consistent observation (2-4, 9, 11, 14). That finding has been associated with an increased number of Kupffer cells (2-4, 6), periportal infiltration of lymphohistiocytic cells (3, 4, 6), and the presence of macrophage-containing granulomas (4, 6, 9, 11). A few investigators (4, 11, 17) have suggested that parenchymal as well as Kupffer cells may be stimulated to proliferate by CP. Substantive information in that regard has not, however, been presented. Should CP administration be shown to result in augmented proliferation of hepatocytes as well as Kupffer cells, there would be reason to assess the effect of CP on liver regeneration following PH. This report presents the results of investigations directed toward determining DNA synthesis, total liver content of DNA, liver weight, and the histology of normal and regenerating livers from animals receiving CP. These studies have been carried out to determine the extent to which hepatocyte proliferation is responsible for the observed hepatomegaly.

MATERIALS AND METHODS

Experimental Animals. Adult Sprague-Dawley female rats weighing 200 to 250 g were used in all experiments. They were housed in individual cages and fed laboratory chow and water *ad libitum*.

CP. CP (Burroughs Wellcome CN6134), supplied by Dr. John K. Whisnant (Burroughs Wellcome & Co. Research Triangle Park, N. C. 27709), was administered via the femoral or tail vein as was 0.9% NaCl solution in control rats. The dose of CP is expressed as mg (dry weight) of organisms in 0.5 ml of suspension.

BCG. BCG was purchased from the Research Foundation, Chicago, Ill. Preparations which consisted of lyophilized live organisms were reconstituted in sterile distilled water and used immediately. The dose used was 1.0 mg (equivalent wet weight) injected i.p., and animals were sacrificed at intervals following injection.

Tilorone Hydrochloride (Tilorone). Tilorone, supplied by Dr. Alfred Richardson, Jr. (Merrell-National Laboratories, Cincinnati, Ohio 45215), was dissolved in 0.9% NaCl solution so that the desired amount, 5 mg/kg, was contained in 0.001 ml/g body weight. It was injected i.p. 5 times/week, Monday through Friday, and animals were sacrificed at intervals following the fifth dose.

Levamisole. Levamisole (*l*-tetramisole), supplied by Dr. E. W. Cantrall (Lederle Laboratories, Pearl River, N. Y. 10965), was prepared immediately before use. It was dissolved in sterile 0.9% NaCl solution so that the desired amount, 5 mg/kg body weight, was contained in 0.001 ml/g body weight. It was injected i.p. every 2 days for 3 doses, and animals were sacrificed at intervals following the third dose.

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[3] The abbreviations used are: CP, *Corynebacterium parvum;* PH, partial hepatectomy; BCG, *Mycobacterium bovis* (strain *Bacillus Calmette-Guérin*); [^{3}H]dThd, tritiated thymidine.

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DNA Synthesis ([³H]dThd Uptake). [³H]dThd (10 μCi; specific activity, 3 Ci/mmol) was injected into the femoral vein 1 hr before sacrifice. The liver was removed, blotted free of blood, and weighed, and a portion was frozen immediately on dry ice. DNA was extracted from the liver with hot 5% trichloroacetic acid (16), and the extract was assayed for DNA content with *p*-nitrophenylhydrazine (18). Radioactivity was determined with a Packard Tri-Carb Model 2425 scintillation spectrometer. Activity is expressed as dpm.

PH. PH was performed by the technique described by Higgins and Anderson (5). In a preliminary series of animals sacrificed immediately after PH, the median and left lateral lobes were found to represent 71.3% of the total liver mass. Administration of CP did not alter the relative weight of these lobes. Following PH the removed lobes were immediately blotted and weighed. The weight of the total liver and of the remnant at operation were calculated from this value. The percentage of liver regeneration was calculated by the following formula:

$$\%\text{ of liver regeneration} = \frac{\text{Sacrifice wt} - \text{Remnant wt at PH}}{\text{Wt removed at PH}} \times 100$$

In this article, the percentage of liver regeneration is synonymous with the percentage of weight restoration.

Histological and Autoradiographic Studies. Portions of liver were obtained from at least 5 normal animals at 1, 2, 3, 4, 5, 7, 8, 9, 14, 17, 28, 35, and 56 days after CP administration, fixed in 10% formalin containing 1% calcium, sectioned at 4 μm, and stained with hematoxylin and eosin in the conventional manner. Those utilized for microautoradiography were deparaffinized, hydrated, and then coated with NTB-2 emulsion (Kodak). These were exposed for 6 to 9 weeks in an air-evacuated, dark container. They were developed with D-19 (Kodak) and stained with Harris' hematoxylin.

Histological sections were similarly prepared from livers obtained from animals at 1, 2, 7, and 14 days following PH, in which animals CP had been administered either 1 or 7 days prior to or at the time of PH.

Experimental Design. In experiments evaluating the effect of CP on normal liver, rats were paired according to weight at the time of injection. One rat in each pair received CP, and the other, 0.5 ml of 0.9% NaCl solution. Pairs of rats were sacrificed at intervals after injection, at which time liver weight, DNA content, and [³H]dThd uptake were determined. In an initial experiment, a single dose of 3.5 mg of CP was used, and rats were sacrificed at intervals from Days 1 to 56 following injection. The effect of various amounts (0.09 to 3.5 mg) of CP was evaluated at 3 and 5 days following injection. Results relative to multiple doses of CP were also obtained. In one such experiment, a second or third dose of CP (3.5 mg) was administered at weekly intervals, and in a subsequent experiment, there was a 4-day interval between the first and second dose.

A second group of investigations evaluated the effect of CP administration on regeneration of rat liver following PH. In those studies, CP (3.5 mg i.v.) was administered either 1 or 7 days prior to or 1 day after PH. Animals were sacrificed at intervals from 1 to 14 days following PH, and the liver weight, percentage of regeneration, DNA synthesis ([³H]dThd uptake), and DNA content of the livers were determined.

Statistics. Statistical analysis of data in Tables 3 and 4 was carried out using a 2-way analysis of variance. Data in Charts 3 and 4 were subjected to analysis using the Student *t* test.

RESULTS

Effect of CP on Intact Liver. Following a single administration of CP (3.5 mg), DNA synthesis, total DNA content, and the weight of the liver increased (Chart 1). By the fourth day following inoculation of CP, the DNA synthesis had increased more than 10-fold [1303 ± 133% (S.E.)] over that in livers from 0.9% NaCl solution-injected controls ($p < 0.001$). Synthesis of DNA sharply declined over the next 4 days and gradually returned to the control level by 56 days. Following CP administration, total DNA content of the liver was elevated and the increase was maximal at Day 7 (196 ± 8%; $p < 0.001$). Although subsequently falling below this value, it remained elevated during the entire period of observation. By the second day following inoculation, the liver weight was 111 ± 4% of that found in controls and continued to increase to a maximum of 143 ± 9% at 9 days ($p < 0.001$). It then slowly decreased in weight. By Day 56, it was still greater (119 ± 6%) than the value for controls ($p = 0.003$).

One day following CP administration to intact animals, the liver exhibited a scant, mixed-lymphoid, and histiocytic infiltrate principally but not exclusively in peripheral and portal zones of lobules (Fig. 1). This reaction appeared to

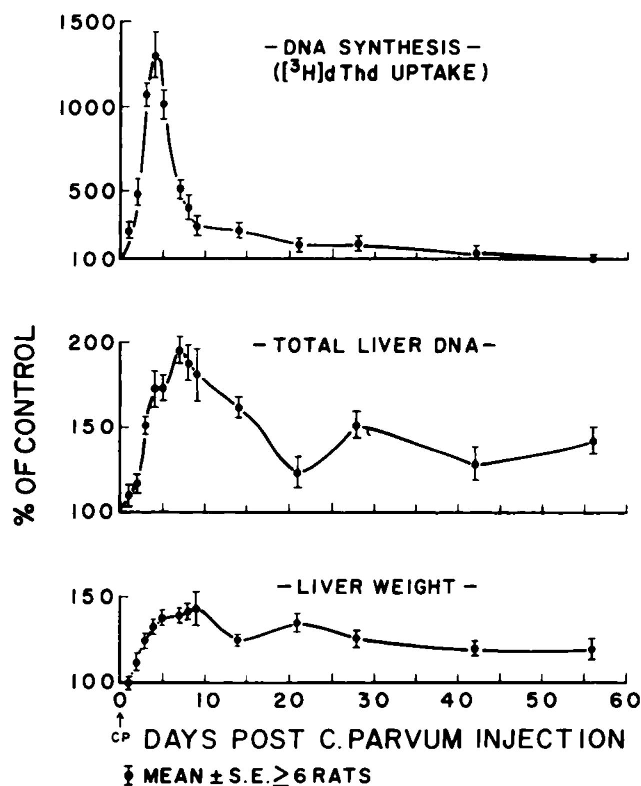

Chart 1. Effect of a single injection of CP on liver weight, DNA synthesis, and total DNA.

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progress so that, at 3 days, discrete foci of necrosis of hepatic cells without zonal predilection were evident. Kupffer cells in such areas and to a lesser degree in uninvolved sites appeared prominent. Intrahepatic branches of the portal veins also exhibited mild endophlebitis. At 4, 5, and 7 days following CP administration, necrotic foci were less prevalent (Fig. 2). Instead, "hard" granulomas characterized by central collections of large histiocytes including some in multinucleated giant cell forms and a peripheral lymphoid histiocytic collar of varying degree were prominent. More mitoses (≈2/high-power field) of hepatocytes were observed during this period than were encountered in sections of livers from rats receiving 0.9% NaCl solution only. Sections obtained at 8 to 56 days following CP administration were qualitatively similar, revealing smaller granulomas and less lymphoid and histiocytic infiltrates (Fig. 3). Quantitatively, this alteration diminished with time so that, at 56 days, only a few such lesions could be recognized and in some instances the liver appeared structurally unaltered.

Significant numbers of grains were observed over nuclei of hepatic and Kupffer cells as well as in those of the inflammatory infiltrate at 4 days following CP administration to intact rats (Fig. 4). Approximately 70% of the labeled cells were identified as hepatocytes.

To assess the effect of varying doses of CP, animals were given a single i.v. injection ranging between 0.09 and 3.5 mg and were sacrificed 3 or 5 days following inoculation. It was observed (Chart 2) that [^{3}H]dThd uptake, total liver DNA, and liver weight were increased by doses of CP ≥ 1.25 mg ($p < 0.05$; except liver weight 3 days after 3.5 mg CP). Although [^{3}H]dThd uptake seemed to be progressively greater 3 days (but not 5 days) after successively larger doses of CP, increases in total liver DNA and liver weight were similar at either 3 or 5 days following the use of CP ≥ 1.25 mg. The histological changes produced by the various doses of CP were quantitatively less severe with lesser amounts but were qualitatively similar. When smaller amounts of CP (<0.09 mg), which failed to produce necrosis, were administered, numerous mitoses were observed in hepatocytes 3 to 5 days following its administration.

When multiple (2 or 3) doses of CP (3.5 mg) were administered at weekly intervals, it was noted (Chart 3) that the second or third dose failed to stimulate DNA synthesis to the same degree as did the first administration. Whereas 4 days after the first dose of CP (the time of maximal stimulation) the [^{3}H]dThd uptake of the entire liver was $1.7 \pm 0.11 \times 10^6$ dpm ($p < 0.001$ compared to control), at a similar time following a second dose it was $0.7 \pm 0.10 \times 10^6$ dpm, and after the third dose it was $0.7 \pm 0.40 \times 10^6$ dpm ($p = 0.2$ and $p < 0.001$, respectively, compared to [^{3}H]dThd uptake at the day of final CP injection). The first dose of CP resulted in a 59% increase in liver DNA and a 31% increase in liver weight 4 days after the CP administration ($p < 0.001$). Increases in total liver DNA and liver weight were found to occur also after the second or third dose of CP, but those increases were not proportionally as great as were the increases following the first dose. Four days after the second dose of CP, the increase in liver DNA was 18% ($p = 0.004$) and the increase in liver weight was 21% ($p < 0.001$) above these values at the time the second dose was

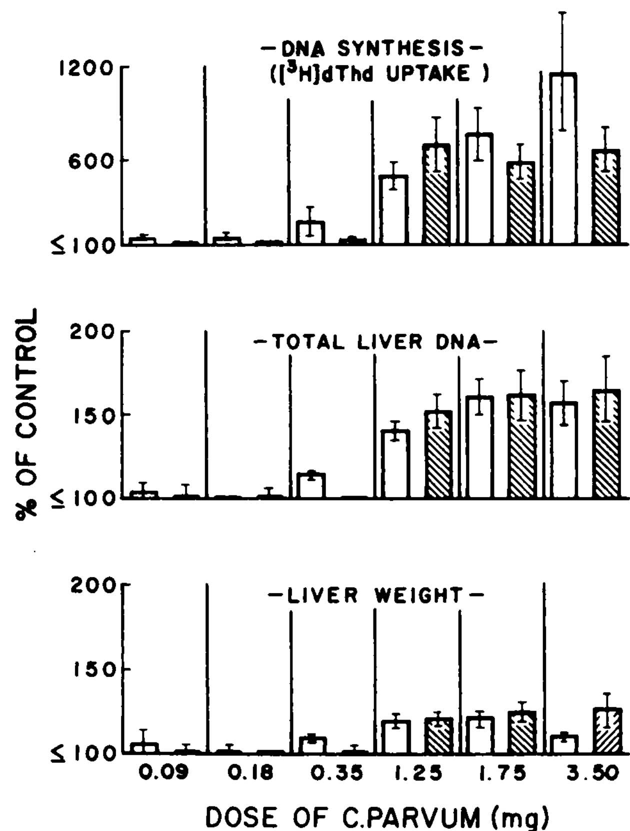

Chart 2. Effect of different doses of CP on liver weight, DNA synthesis, and total liver DNA. *Bars*, ±S.E. of 3 to 5 rats/group; □, 3 days post-CP; ▧, 5 days post-CP.

given. Similar values 4 days after the third dose were 28 ($p = 0.003$) and 20% ($p = 0.02$), respectively. An additional experiment was carried out to determine whether a second dose of CP administered at the time of peak DNA synthesis would augment the effect of the first dose (Chart 4). No further increase in [^{3}H]dThd uptake resulted. There was, however, a slower decline toward normal levels. This finding was reflected in the observation that both total liver DNA and liver weight were slightly increased above that occurring as a result of the first dose. There was a 14% increase in weight 4 days following the second dose ($p = 0.004$), in contrast to a 31% increase 4 days following the first dose ($p < 0.001$).

No qualitative difference in the liver was observed histologically following 1, 2, or 3 doses of CP. Moreover, the appearance of the liver was similar whether the second dose of CP was given 4 or 7 days after the first dose.

The effect of CP on DNA synthesis, total liver DNA, and liver weight was compared to that of several other immunomodulators, 1 microbial (BCG) and 2 chemical (tilorone and levamisole). It was observed (Table 1) that both of the microbial agents produced a comparable effect on DNA synthesis. The peak uptake, however, with BCG occurred 8 days following BCG in contrast to 4 days following CP. This was reflected in a similar increase in total liver DNA and liver weight following the use of these agents. Whereas tilorone had an effect which was less than that following CP or BCG, levamisole produced no effect. Tilorone and levamisole resulted in no histological changes. The effects of

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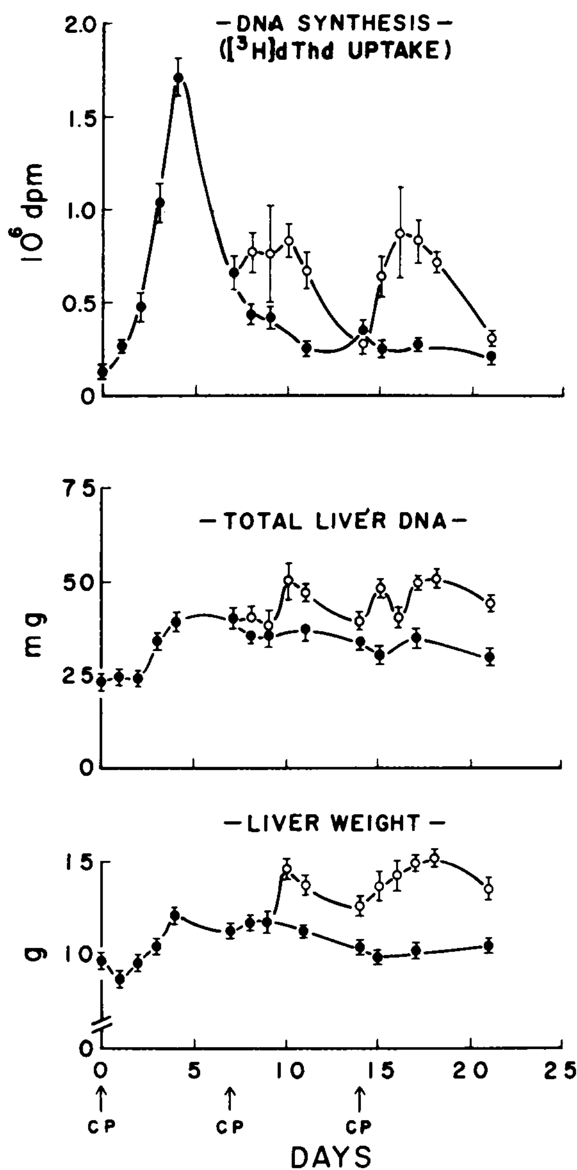

Chart 3. Effect of multiple doses of CP on liver weight, DNA synthesis, and total DNA. *Bars*, ±S.E. of 6 rats; ●, CP (3.5 mg for 1 dose); ○, CP (3.5 mg every 7 days for 2 or 3 doses).

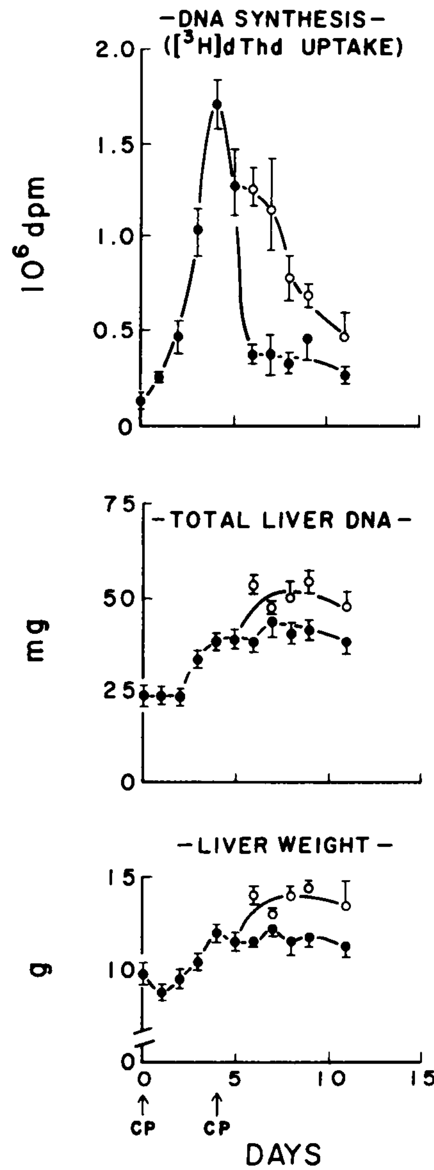

Chart 4. Effect of a second dose of CP given at the peak of DNA synthesis on liver weight, DNA synthesis, and total DNA. *Bars*, ±S.E. of 6 rats; ●, CP (3.5 mg, Day 0); ○, CP (3.5 mg, Days 0 and 4).

Table 1

Effect of various immunomodulators on DNA synthesis, total liver DNA, and liver weight

	Maximum [³H]dThd uptake			Maximum total liver DNA			Maximum liver wt		
	Day post-injection	10^5 dpm	No. of rats	Day post-injection	mg/liver	No. of rats	Day post-injection	g	No. of rats
CP	4	19.10 ± 1.59[a]	11	5	40.5 ± 1.59	13	5	12.0 ± 0.24	16
BCG	8	12.89 ± 1.22	3	8	37.9 ± 0.40	3	8	11.5 ± 0.27	3
Levamisole	7	1.48 ± 0.20	3	3	26.6 ± 0.96	3	3	9.5 ± 0.14	3
Tilorone	5	9.28	2	7	31.0	2	7	12.0	2
0.9% NaCl solution		1.20 ± 0.10	129		21.9 ± 0.29	129		8.4 ± 0.08	133

[a] Mean ± S.E.

BCG were similar to those of CP but appeared after a longer interval following administration.

To assess the relationship of liver damage to the changes observed with CP, animals were given a single injection (1.6 mg/kg) of chloroform in mineral oil and were sacrificed at intervals. Despite evidence of severe central lobular necrosis and mortality (5 of 15 died), little increase in total liver DNA or liver weight was observed (Table 2). An increase in

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[^{3}H]dThd uptake was observed 2 days after chloroform injection.

Effect of CP on Regenerating Liver following 70% Hepatectomy. When CP (3.5 mg) was administered 7 days prior to 70% PH, it was observed that liver weight at the time of PH was greater in treated than in control animals (Table 3).

Table 2

Effect of chloroform on DNA synthesis

Days postchloroform	Total liver DNA and liver wt		
	[^{3}H]dThd (10^5 dpm)	Total liver DNA (mg)	Liver wt (g)
1	1.86 ± 0.34[a]	23.9 ± 1.05	9.1 ± 0.51
2	12.77 ± 0.60	21.9 ± 0.52	8.8 ± 0.42
4	2.25 ± 0.27	24.6 ± 0.73	8.2 ± 0.63
14	0.86 ± 0.04	18.9 ± 0.66	9.2 ± 0.56

[a] Mean ± S.E. of 3 rats in each observation.

The percentage of regeneration was less in the treated animals than in their respective controls, although liver weight at sacrifice remained greater in the CP-treated animals between 7 and 14 days posthepatectomy. When CP was administered 1 day prior to hepatectomy, the percentage of regeneration was greater at all days in the treated animals. At all time intervals, liver weights at sacrifice were greater in the CP-treated animals. Similar results were observed when CP was given 1 day after PH. Seven and 14 days following PH, regeneration was >100% in treated rats, and liver weights at sacrifice were greater than were those of control animals.

The administration of CP (3.5 mg) 7 days prior to PH had no significant effect on DNA synthesis 1 and 2 days following PH (Table 4). At 7 and 14 days following PH, total liver DNA synthesis was significantly increased in animals that received CP. The total DNA content of livers from CP-

Table 3

Effect of CP on regenerating liver following 70% hepatectomy (PH): liver weights and percentage of regeneration

Time of CP relative to PH	Sacrifice time following PH	Total liver wt (g)				% of regeneration	
		At PH		At sacrifice			
		Control	CP treated	Control	CP treated	Control	CP treated
−7	1	9.7 ± 0.31[a]	13.4 ± 0.31[b]	4.0 ± 0.13	7.2 ± 0.40[b]	16.9 ± 1.88	9.7 ± 1.97[b]
	2	9.3 ± 0.52	13.6 ± 0.63	5.5 ± 0.20	7.0 ± 0.17	40.7 ± 2.27	31.0 ± 4.64
	7	8.1 ± 0.48	13.2 ± 0.42	6.6 ± 0.36	8.1 ± 0.26	71.7 ± 3.34	42.9 ± 2.41
	14	8.7 ± 0.34	11.9 ± 0.27	7.9 ± 0.34	8.2 ± 0.11	86.5 ± 4.01	55.6 ± 2.40
−1	1	8.3 ± 0.34	8.1 ± 0.35	3.6 ± 0.10	4.1 ± 0.16[b]	19.2 ± 1.75	29.3 ± 3.85[b]
	2	9.2 ± 0.21	9.0 ± 0.22	5.2 ± 0.22	6.5 ± 0.17	39.3 ± 1.54	61.6 ± 3.74
	7	8.5 ± 0.26	8.3 ± 0.45	7.7 ± 0.23	9.0 ± 0.38	87.0 ± 6.45	113.3 ± 10.33
	14	8.4 ± 0.39	8.6 ± 0.21	7.7 ± 0.31	9.6 ± 0.30	90.0 ± 3.54	115.0 ± 5.85
+1	1	10.1 ± 0.22		4.0 ± 0.14		13.7 ± 1.40	
	2	10.4 ± 0.16	10.3 ± 0.29	6.0 ± 0.16	5.9 ± 0.06	39.1 ± 2.39	38.1 ± 0.73
	7	9.5 ± 0.33	10.2 ± 0.43	7.7 ± 0.24	10.8 ± 0.41	73.3 ± 6.45	106.6 ± 1.27
	14	10.5 ± 0.46	10.5 ± 0.63	9.2 ± 0.26	11.4 ± 0.65	82.9 ± 7.59	114.1 ± 10.08

[a] Mean ± S.E. of 6 rats.
[b] Significant at 0.01 level.

Table 4

Effect of CP on regenerating liver following 70% hepatectomy (PH): DNA synthesis ([^{3}H]dThd uptake) and liver DNA content

Time of CP relative to PH	Sacrifice time following PH	Total liver DNA synthesis ([^{3}H]dThd uptake) at sacrifice (10^5 dpm)		Total liver DNA (mg) at sacrifice	
		Control	CP treated	Control	CP treated
−7	1	17.43 ± 4.72[a]	9.72 ± 2.41	10.50 ± 0.73	14.7 ± 0.74[b]
	2	10.92 ± 1.32	12.1 ± 1.06	14.33 ± 0.49	21.66 ± 1.11
	7	1.43 ± 0.17	2.00 ± 0.14	20.66 ± 1.11	23.50 ± 1.72
	14	1.31 ± 0.15	1.98 ± 0.12	21.50 ± 0.88	23.67 ± 0.76
−1	1	13.07 ± 2.63	21.39 ± 2.97[b]	8.81 ± 0.63	10.42 ± 0.42[b]
	2	10.74 ± 1.29	12.39 ± 0.82	12.66 ± 0.55	19.66 ± 0.76
	7	1.45 ± 0.14	3.88 ± 0.52	21.70 ± 0.80	30.80 ± 1.24
	14	1.43 ± 0.17	1.88 ± 0.21	20.20 ± 1.49	26.00 ± 1.78
+1	1	19.16 ± 1.82		10.17 ± 0.05	
	2	10.98 ± 1.77	12.50 ± 0.92[c]	15.15 ± 0.47	15.50 ± 0.70[b]
	7	1.16 ± 0.23	4.58 ± 1.44	19.94 ± 0.63	36.10 ± 1.74
	14	1.61 ± 0.33	3.11 ± 0.68	23.04 ± 1.71	33.03 ± 2.66

[a] Mean ± S.E. of 6 rats.
[b] Significant at 0.01 level.
[c] Significant at 0.05 level.

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treated animals was greater than that of controls 1 and 2 days following PH. By Day 7, DNA content was similar in both groups.

When CP was given 1 day prior to PH, total liver DNA synthesis was greater in treated than control animals, although this difference was statistically significant only 7 days after PH. The peak of DNA synthesis occurred 24 hr after PH in treated and untreated animals. Total liver DNA was greater at all days following PH in animals receiving CP than in those given 0.9% NaCl solution. CP administered 1 day after PH resulted in findings relative to DNA synthesis and total liver DNA which were essentially similar to those occurring when CP was given 1 day prior to PH.

Hepatic alterations in livers obtained from rats subjected to PH and CP administration were chronologically similar both quantitatively and qualitatively to those observed in the intact animals described above. As might be expected, mitoses were more frequent in hepatocytes of hepatectomized animals, particularly 2 days following operation.

DISCUSSION

The presently reported histological and morphological changes in rat livers following CP administration are similar to those noted in mice. Reticuloendothelial proliferation (2–4, 6, 11), granuloma formation (4, 6, 9, 11), hepatomegaly (2–4, 9–11), and focal parenchymal necrosis (2, 3, 9) have been described. Our long-standing interest in hepatic regeneration has directed our attention to a consideration of mechanism(s) responsible for the hepatomegaly resulting from CP. Increased liver size has for the most part been ascribed to the lymphohistiocytic infiltrate which occurs (4, 17). The few investigators who have noted hepatocellular proliferation (4, 11, 17) following CP administration have not specifically associated that finding with the hepatomegaly produced. Halpern *et al.* (4) described an increase in the number of binucleated hepatocytes, McBride *et al.* (11) mentioned that there was autoradiographic evidence of parenchymal cell proliferation, and Sljivić and Warr (17) found that liver parenchymal cells were labeled with [^{3}H]dThd. It has also been noted that saccharated iron oxide (Proferrin), a reticuloendothelial stimulant, produced increased hepatic DNA synthesis, and autoradiography revealed that a "sizable fraction" of this DNA synthesis was due to parenchymal cell proliferation (8). The effect of CP appears to be strain related since at least 1 strain (10387) failed to cause hepatomegaly even though granuloma formation was present (12).

The presently reported findings indicating that, 4 days following a single dose of CP, DNA synthesis was at a maximum and that autoradiography at that time revealed that ≃70% of the labeled cells were hepatocytes denotes that parenchymal cell proliferation substantially contributes to the elevated [^{3}H]dThd uptake. Thus increased liver weight following administration of CP is related to hepatocyte proliferation as well as lymphohistiocytic infiltration. The associated increase in total liver DNA excludes the possibility that the hepatomegaly was solely the result of increased content of hepatic fat, glycogen, or increased water content.

In view of the findings indicating that CP administration resulted in hepatocyte proliferation which contributed to the hepatomegaly, it was pertinent to determine whether CP augmented liver regeneration following PH. The present findings are the first to indicate that CP has such an effect. This was most striking when the CP was administered in proximity to the time of hepatectomy, *i.e.*, 1 day prior to or 1 day after liver removal. Similar to findings observed in nonhepatectomized livers, the increased liver DNA is indicative of active cellular proliferation.

When CP administration preceded PH by 7 days, the liver remnant at PH was markedly larger than that left behind in non-CP-treated controls. Consequently, although the increment in liver mass in the CP and control groups was found to be similar at sacrifice, the percentage of regeneration was lower in the former group, *i.e.*, CP treated. Such findings are in keeping with those over the years, indicating that the larger the liver remnant, the smaller the amount of liver regenerated (1, 7). They also indicate that the larger remnant in the CP-treated animals consisted of an increased number of hepatocytes, for should the greater size have been related substantially to nonparenchymal cells, *e.g.*, cell infiltrate, a regenerative response at least equivalent to controls would have been anticipated. Findings relative to liver DNA and those indicating that [^{3}H]dThd uptake 24 hr following PH was less in treated than control livers are in keeping with those regarding liver mass at hepatectomy. Although such an explanation seems most appropriate to account for the reduced percentage of regeneration in the CP-treated rats, the possibility exists that CP may inhibit hepatic regeneration when administered at certain times relative to PH.

The mechanism(s) responsible for the stimulation of hepatocyte replication are conjectural. That liver necrosis subsequent to CP administration may initiate a regenerative response to replace damaged cells is a possibility. For several reasons, however, it seems unlikely that the hepatomegaly is entirely a result of parenchymal cell stimulation consequent to liver damage. Evidence exists indicating that chloroform in a dose which produces central lobular necrosis, significant mortality, and increased [^{3}H]dThd uptake 2 days following its administration failed to lead to an increase in liver weight or DNA content. In addition, the finding indicating that an amount of CP which produces little hepatic necrosis does result in an increase in hepatocyte mitoses further limits the significance of liver damage as the only factor responsible for the parenchymal cell proliferation. Finally, if cellular necrosis served as a stimulus for parenchymal proliferation, it might have been anticipated that, in contrast to what was observed, each dose of CP would have been accompanied by at least a comparable rise in [^{3}H]dThd uptake. It may be postulated that CP directly stimulates resting (G_0) hepatocytes so that they undergo DNA synthesis and mitosis and/or stimulates G_2 cells so that they undergo mitosis. The findings that CP, BCG, and (to a lesser degree) tilorone result in hepatocellular proliferation and may enhance liver regeneration are of particular interest in view of information by others suggesting that immune mechanisms may play a role in liver regeneration (13, 15).

Since Halpern *et al.* (4) have noted and the present histological findings confirm that CP does not result in

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permanent liver parenchymal damage or fibrosis, further investigations seem indicated to assess the possible worth of CP administration in those situations in which the liver has been compromised and augmented regeneration might be of benefit.

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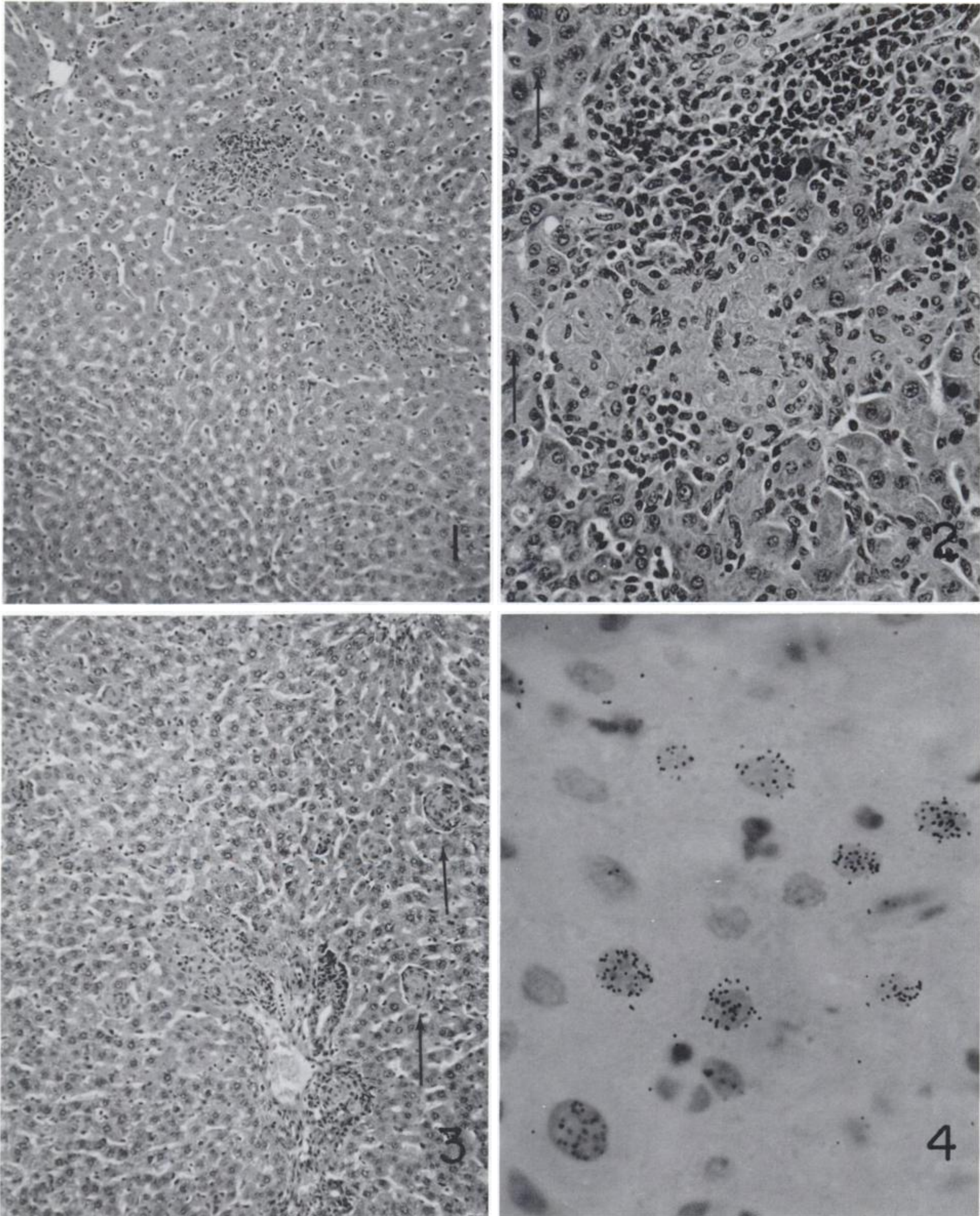

Fig. 1. Area of liver from intact rat 2 days after administration of CP. There is focal lymphoid infiltrate present. H & E, × 100.

Fig. 2. Focus of necrosis in liver from intact rat treated with CP 5 days previously. Several mitoses in hepatocytes adjacent to this area are present (*arrows*), and Kupffer cells appear larger than usual. H & E, × 250.

Fig. 3. Discrete, "small, hard" granulomas (*arrows*) noted in liver of intact rat 14 days after receiving CP. H & E, × 100.

Fig. 4. Microautoradiograph of liver from intact rat treated 4 days previously with CP. Labeling of hepatocyte nuclei is conspicuous. Harris' hematoxylin, × 400.

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