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DNA–Anthracycline Complexes. II. Comparative Study of the Acute Lesions Induced in Mice after Intravenous Administration of Free and DNA Bound Adriamycin

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Absract—*We have compared in mice the overall toxicity of free adriamycin (ADR) or DNA bound adriamycin (ADR–DNA) after repeated i.v. injections of high drug doses* (35–40 mg/kg). *Survival curves and variations in mean total body weight indicated that ADR–DNA is less toxic than ADR in mice. Histopathologic studies showed:* (1) *that ADR induced severe lesions in the hemopoietic organs (bone marrow, lymph nodes, spleen, thymus) and in the gastrointestinal tract. An important lipid overloading in liver and kidney was observed;* (2) *that the toxicity of ADR is reduced for bone marrow and stomach when the drug is associated with high mol. wt DNA. No lipid overloading has been seen in liver and kidney of the complex treated mice.*

The repair of the lesions induced in the tissues (lymphoid organs, thymus) which are not protected against the effect of ADR when using a DNA-complex, is significantly more rapid. Finally, in this subacute study in mice, no cardiomyopathy has been observed after treatment with either free or DNA bound drug.

INTRODUCTION

ADRIAMYCIN (ADR) is an anthracycline antibiotic widely used in the treatment of neoplastic diseases in man, however several manifestations of toxicity limit its administration [1, 2].

We have previously described [3] that the lethality of ADR in mice can significantly be reduced when the drug is administered as a complex with high mol. wt DNA. Jaenke (unpublished results) has found that in the rabbit, the myocardial toxicity of ADR–DNA is significantly lower than that of ADR. Moreover clinical data [4–8] obtained in patients treated with ADR–DNA suggest that the cardiotoxicity of ADR can significantly be reduced.

To compare the overall toxicity of free or DNA bound ADR, we have conducted histopathologic studies in mice after repeated intravenous injections of high doses of free or complexed ADR.

MATERIALS AND METHODS

Female DBA_2 mice (Charles River, France) of 20–22 g were used. ADR, free (Farmitalia, Milan) or bound to DNA, extracted from herring sperm (type VII, Sigma Chemicals, St Louis, Missouri, U.S.A.) was given i.v. The ADR–DNA complex was prepared as described previously [3], using a nucleotide/drug molar ratio of 20. In a first experiment (series

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A: morphologic changes) the drugs were given during 5 consecutive days at 7 mg/kg per day. For each drug form, two groups of mice were injected: in the first group, 10 animals were weighed daily and the lethality was recorded until the 25th day after the first injection; the second group was used for the histopathologic study: six mice for the free ADR series and eight mice for the complex series were sacrificed the 10th day following the first drug injection. A second experiment (series B) was undertaken for serial sacrifice: the schedule included four consecutive i.v. injections at 10 mg/kg per day of free or DNA-bound ADR. Ten mice per drug form were followed for weight changes and mortality until the 14th day after the first injection. Other series of five mice receiving a similar dose of each drug were sacrificed respectively on the 6th, 8th and 13th day after the first injection. Control series included at least five untreated mice in each experimental group (weight–mortality–histopathology).

For histopathologic studies, the mice were sacrificed by cervical dislocation and selected tissues were removed for light-microscopic examination. Tissues were fixed in 10% neutral buffered formaldehyde solution and then processed for paraffin embedding. The paraffin slices were stained with hematoxylin–eosin–safran or by the P.A.S. method combined with hematoxylin–safran. Samples of the liver fixed in neutral buffered formaldehyde were also cut with a cryostat and the frozen sections were stained for neutral fats by the oil-red 0 staining method.

RESULTS

In a first part of the experimental results (series A) we describe the morphologic changes induced in mice treated with ADR or ADR–DNA using a daily schedule of 7 mg/kg of ADR on 5 consecutive days. In a second part (series B) we report the progression of the lesions observed in mice treated with ADR or ADR–DNA using a schedule of 4 consecutive daily injections of 10 mg of ADR per kg.

Survival curves (Fig. 1) show that 10 days following the first injection, only 20–40% of animals were alive after administration of ADR whereas 90% survivors were observed when ADR was given associated with DNA.

Examination of variations in mean total body wt (Fig. 2) indicates that mice receiving

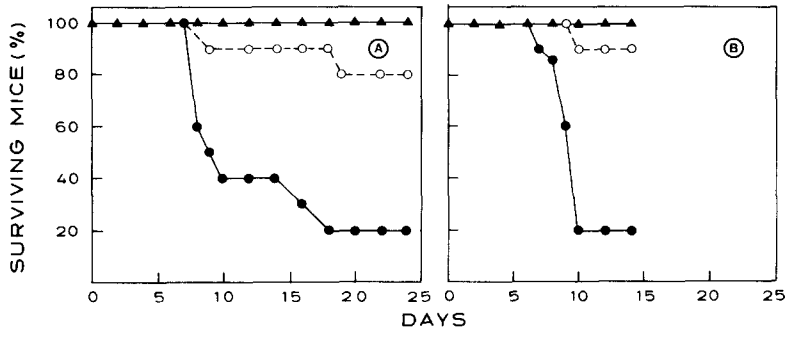


Fig. 1. Survival curves. (A) Treatment schedule; 5 × 7 mg/kg/day. *(B) Treatment schedule:* 4 × 10 mg/kg/day. (▲) *control mice;* (●) *ADR treated mice;* (○) *ADR–DNA treated mice.*

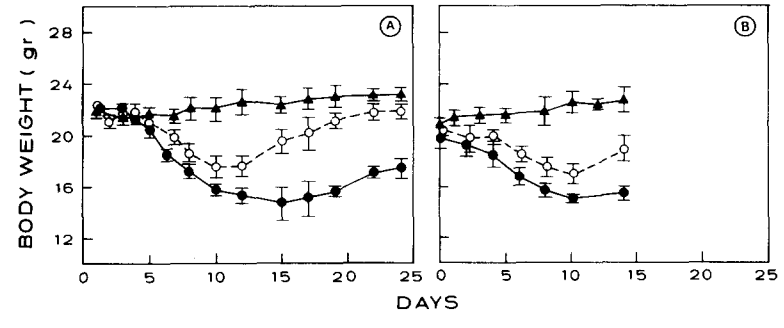


Fig. 2. Body weight changes. (A) Treatment schedule: 5 × 7 mg/kg/day. *(B) Treatment schedule:* 4 × 10 mg/kg/day. (▲) *Control mice;* (●) *ADR treated mice;* (○) *ADR–DNA treated mice. Mean* ±S.D. *of individual values are given.*

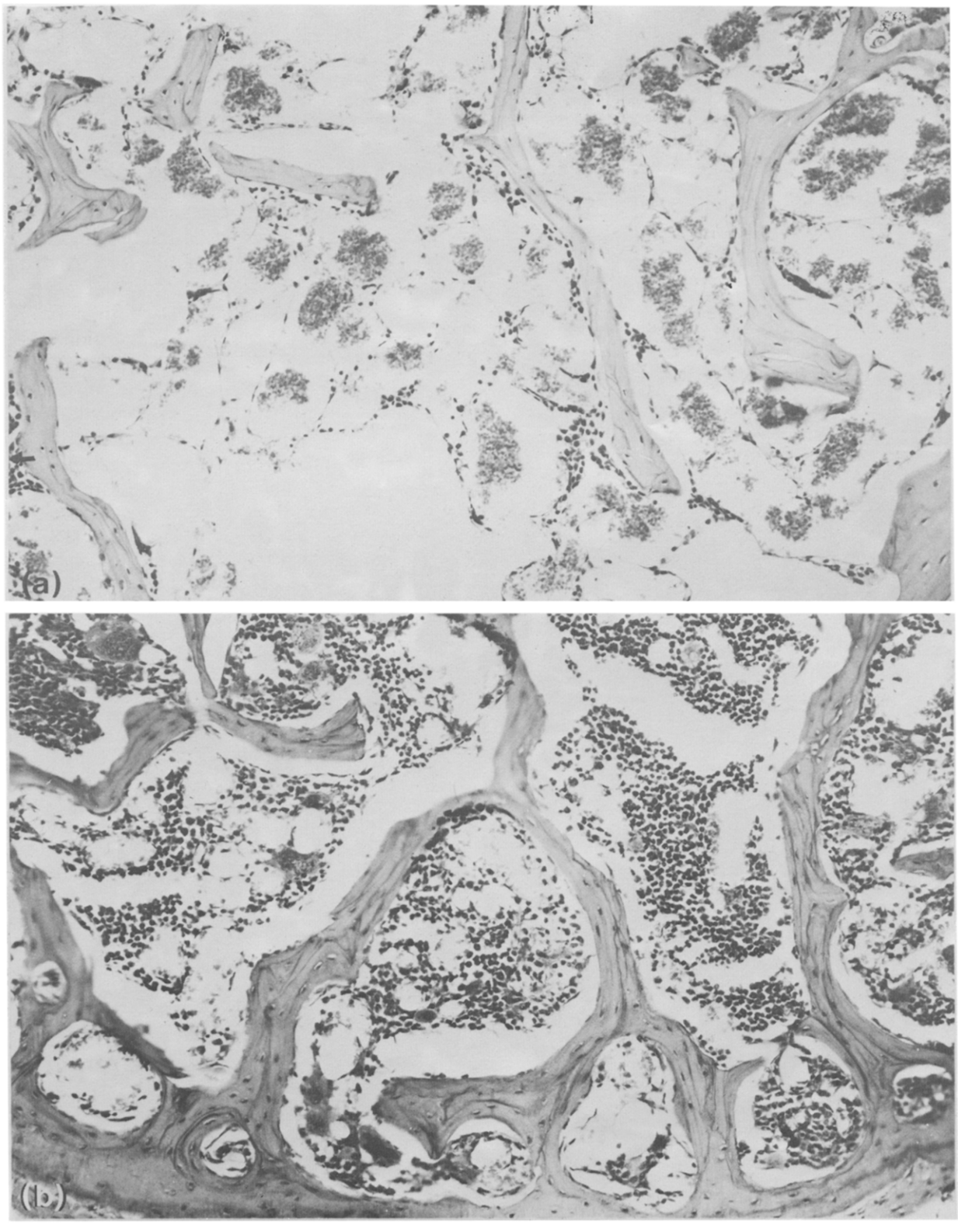


Fig. 3 *Bone marrow.* (*a*) *Adriamycin treated mouse* (× 160). (*b*) *Adriamycin–DNA complex treated mouse* (× 160).

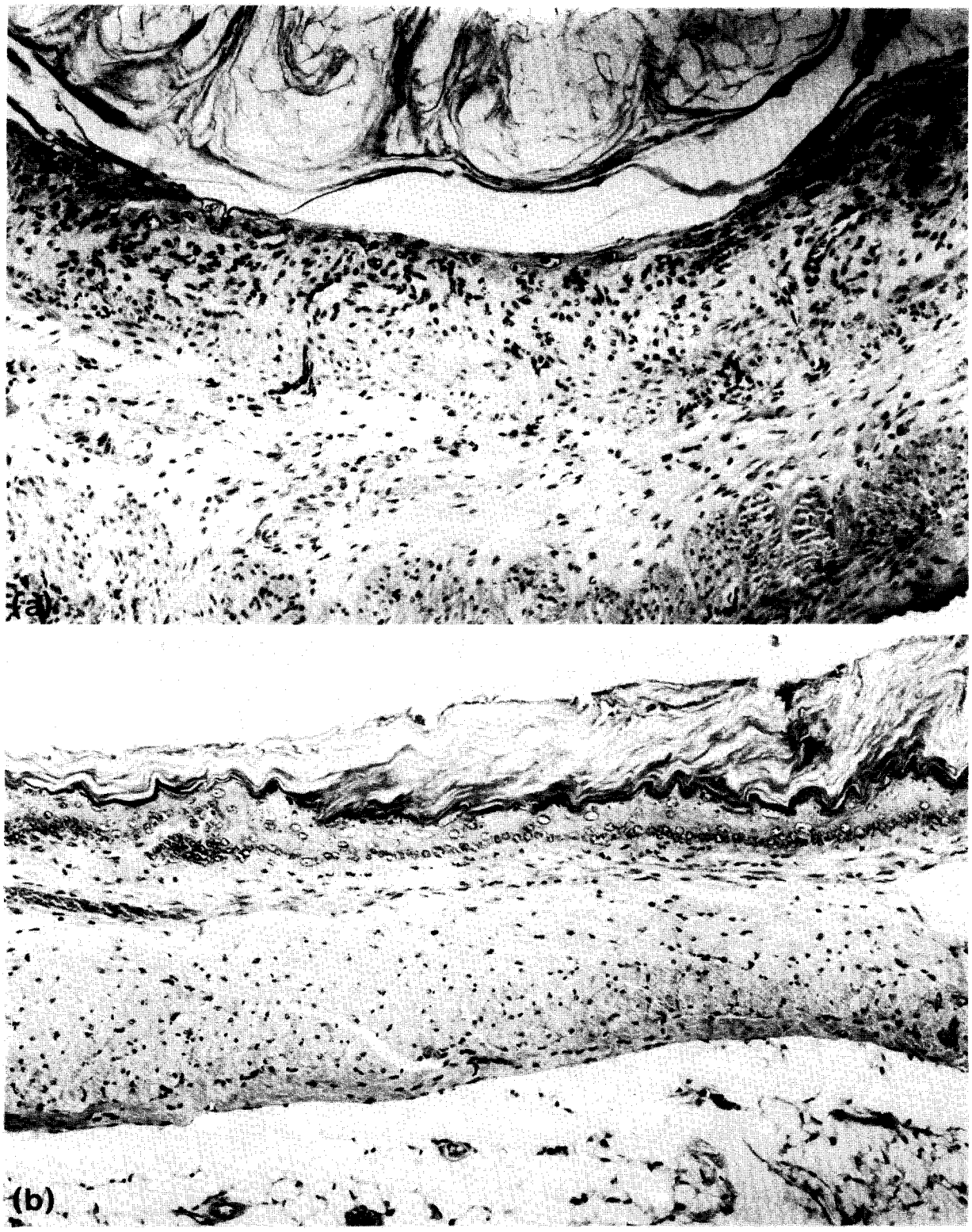


Fig. 4. Nonglandular stomach. (a) Adriamycin treated mouse. Lamina propria at bottom; thin discontinuous cornified epithelium at center (×160). (b) Adriamycin-D.N.A complex treated mouse. More normal stratified squamous epithelial layer (×160).

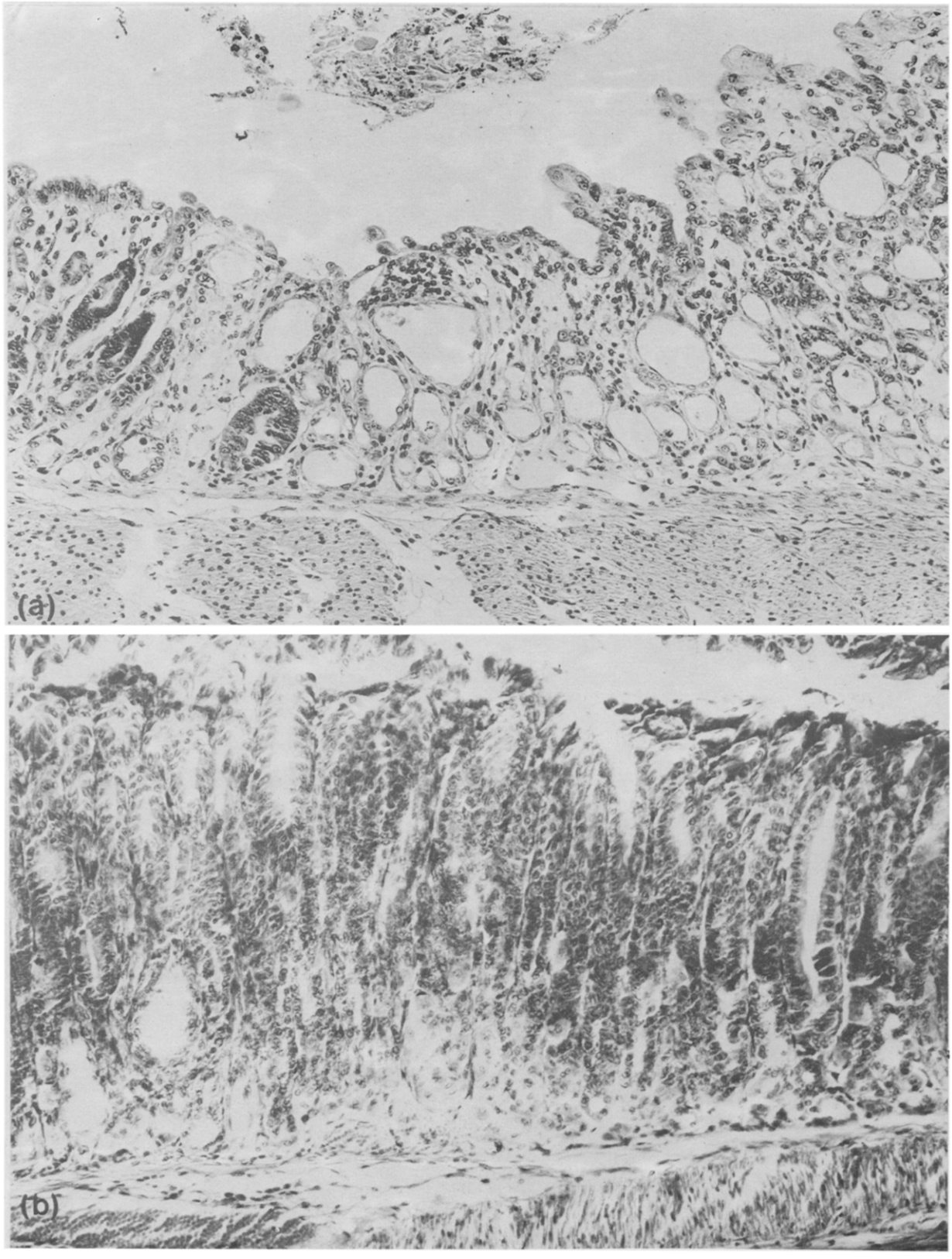
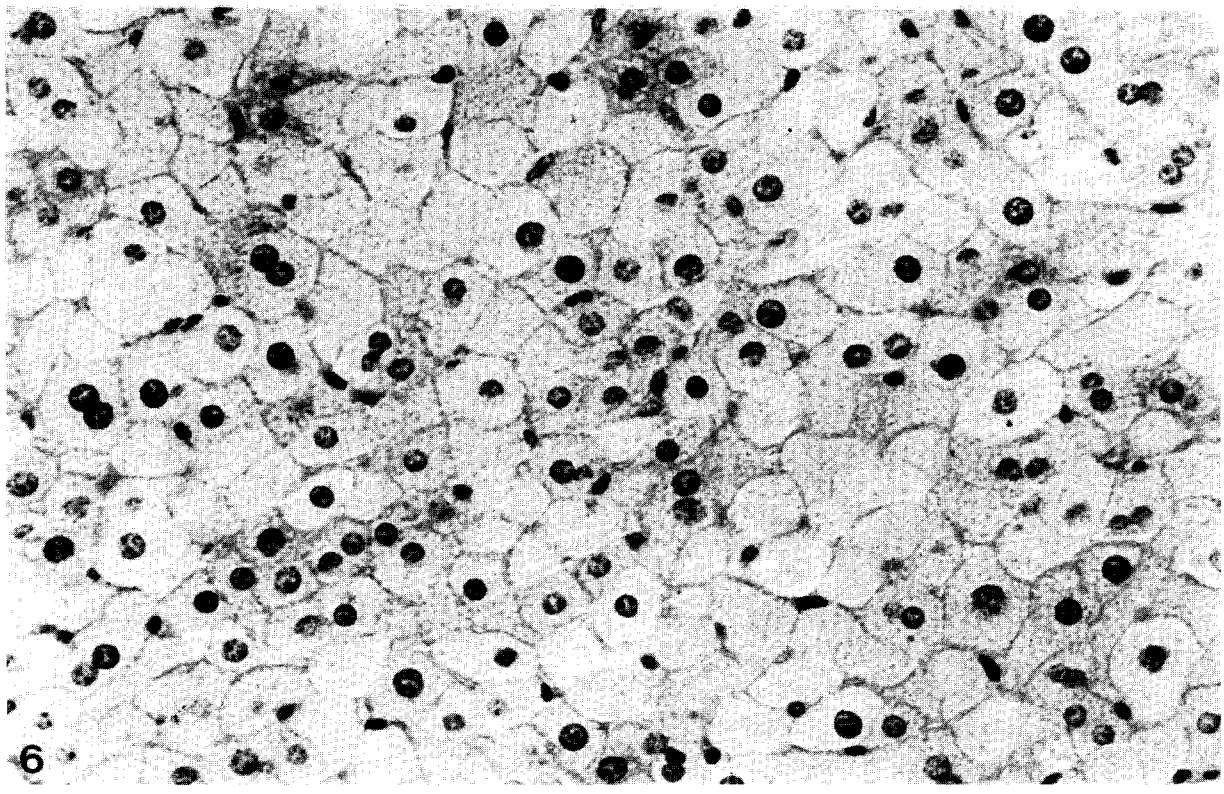


Fig. 5. Glandular stomach. (a) Adriamycin treated mouse exhibiting atrophy and cystic changes (×160). *(b) Adriamycin–D.NA complex treated mouse. Normal stratified squamous epithelial layer* (×160).



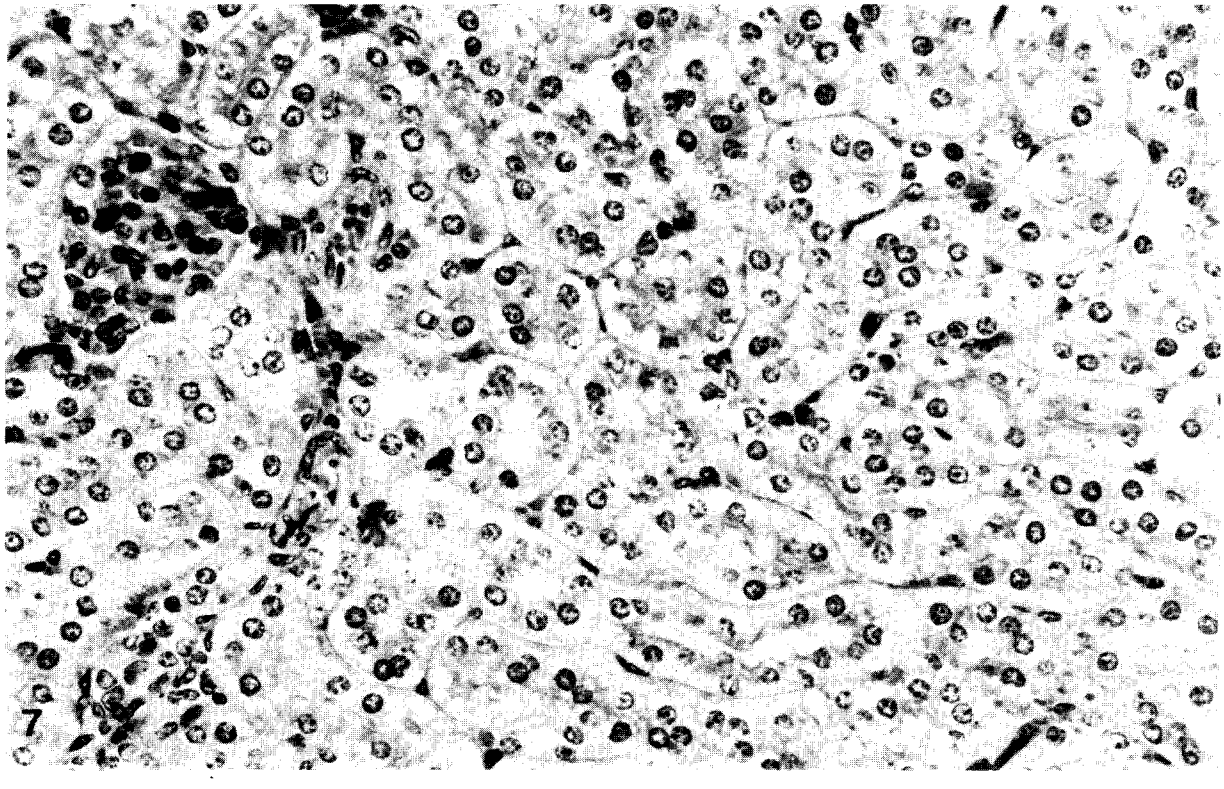


Fig. 6. Liver. Adriamycin treated mouse. Hepatocytes are swollen and contain many fine vacuoles (×400).

Fig. 7. Kidney. Adriamycin treated mouse. Epithelial cells of proximal convoluted tubules contain multiple lipid vacuoles (×400).

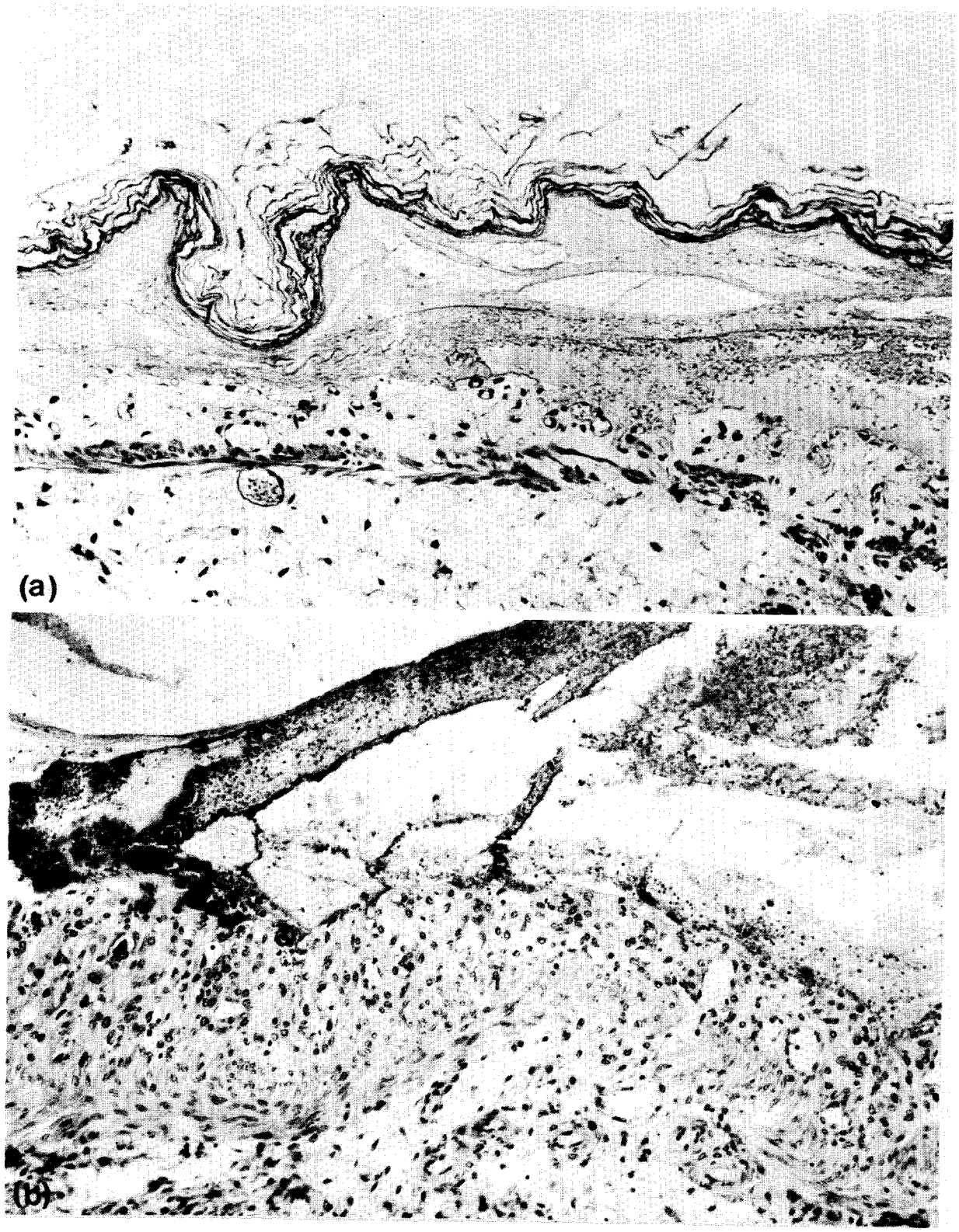


Fig. 8. Nonglandular stomach. (a) Adriamycin treated mouse. Mucosal epithelium (top) is thin; lamina propria distended by edema fluid, erithrocytes and fibrin (×160). *(b) Adriamycin treated mouse. The epithelium is ulcerated and is covered by fibrin and bacteria* (×160).