BIODEGRADATION OF ACETAMINOPHEN

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Abstract

Species of ***Micrococcus, Seiratia, Streptococcus, Bacillus, Escherichia*** and ***Staphylococcus*** isolated from paracetamol syrups were evaluated for potentials to utilize acetaminophen (active ingredient of paracetamol) as a source of carbon and energy. The results revealed that 3 (1 ***Staphylococcus*** sp. and 2 ***Bacillus*** sp.) out of 12 isolates tested, utilized the product at considerably high rates. The viable plate counts and optical densities of the cultures increased while pH values decreased with time. The implications of the results obtained are discussed in relation to microbial contamination of pharmaceutical products and the effects on health.

Introduction

Acetaminophen (N-acetyl-p-aminophenol) is a drug available over- the- counter under the trade names- tylenol, panadol and paracetamol and is also an ingredient in stronger prescription of pain killers such as percocet and darvocet. Acetaminophen is a heat stable compound that belongs to a subgroup of analgesics called salycylates, the rest being narcotic alkaloids and synthetics (Terry, 1991). It is the active component of paracetamol. In most drugs containing acetaminophen it is the active ingredient, the rest being colouring matter, flavours and preservatives.

Paracetamol syrup is cheap and most commonly used for the relief of fever in children. It may be due to this wide patronage that many pharmaceutical companies in Nigeria produce the syrup. The safety of paracetamol and the fact that it is available without prescription make it one of the most widely used drugs for self-medication. It has been established that some 35 tons are consumed daily in the United States alone (Daniel and Lester, 1977). However it has been established that some pharmaceutical preparations used in the treatment of diseases can become contaminated with even pathogenic organisms and therefore, themselves become ineffective and harmful rather and constitutes economic loss. This possibility makes it desirable that the microbiological status of drugs is ascertained before use.

The presence of objectionable microorganisms in pharmaceutical preparations is regarded as health hazards to users because of their potentials to cause infection in man. Earlier study in our laboratory has identified various bacterial contaminants of paracetamol syrups sold in pharmacists' shops in Minna, Niger State, Nigeria. The organisms include species of ***Streptococcus, Bacillus, Micrococcus*** and ***Staphylococcus*** . The present study seeks to evaluate susceptibility of the active ingredient of paracetamol (acetaminophen) to some of the bacterial isolates.

The study will go a long way in informing the manufacturers of the potential danger of the contaminants in terms of immediate loss of product or in the increasing cost of dealing with litigation should the spoilage or breakdown of active ingredient cause harm to the consumers. In response, the manufacturers can therefore, operate under conditions that are detrimental to the survival and growth of such contaminants and devise ways and means of combating the contaminants if the organisms exist on products or raw materials. The aim of this study is to determine the biodegradation of acetaminophen by bacteria isolated from paracetamol syrups.

Materials and Methods Microorganisms

The bacteria used in this study v/ere originally isolated from paracetamol syrups at the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The bacteria are ***Streptococcus*** sp (2), ***Staphylococcus epidermidis*** (1), ***Micrococcus*** sp. (2), ***Bacillus*** sp. (4), ***Staphylococcus aureus*** (1), ***Bacillus sublilis*** (1) and ***Bacillus cereus*** (1). The bacteria were coded for easy identification since more than one of each genus was used .

Screening Test of Bacterial Isolates for Ability to Utilize Acetaminophen

Two hundred millilitres (200ml) of mineral salts **(1.2g KH_2P0_4, 4.0g NH_4C1, 0.2g** $MgS0_4.7H_20$, O.lg NaCl, O.Olg $FeS0_4.7H_20$, pH7.4) distributed into two conical flasks. To the contents of one conical flask was added lg of crystalline acetaminophen powder, heated gently to

dissolve and labeled C. No acetaminophen powder was added to the other flasks. This was labeled P. The content of flasks C was then dispensed into sterile test tubes. The bacterial cells were inoculated in the tubes and all tubes were incubated at room temperature (28 ± 2°C) for 6 days. Tubes from group P acted as controls for tubes from group C. The tubes were visually observed each day for turbidity of the medium as a measure of growth and utilization of acetaminophen.

Biodagradation of Acetaminophen by Bacterial Isolates

This stage of the research was performed to determine the rates of degradation of acetaminophen (2.3%) by isolates, which exhibited maximum growth in acetaminophen medium. Basal medium was prepared with the incorporation of 2.3% crystalline acetaminophen, which was then dissolved by gentle heating. The medium was dispensed into 4 conical flasks (250ml per flask). All flasks were sterilized by autoclaving at 121°C for 15 min. and left to cool to 45°C. One millilitre of each of the selected isolates was seeded from overnight nutrient broth cultures onto one of the flasks. The remaining flasks were labeled as control. All flasks were incubated at room temperature (28+2"C) Ibr 25 days. Flasks were being ascptieally aerated at 24h interval.

Viable plate counts were determined by plating serially diluted samples on Nutrient agar and counting colonies, which developed after 48h. Optical densities were measured, using the control culture as blank reading, at absorbance of 430nm with the Colorimeter (Model 6051, Jenway Limited, Fested, U. K). pH measurements were taken with the pH meter (Micro pH 2000 Crison Instruments, S.A., Barcelona) for 25 days. The parameters were determined at 5 days interval.

Results

Growth of bacterial isolates on acetaminophen

The results of the rate of utilization of acetaminophen by bacteria are presented in Table 1. The results revealed that the isolates utilized the chemical at varying rates. Six out of 12 isolates were able to utilize acetaminophen. Three isolates exhibited considerably high ability in utilizing the compound while only one isolate utilized the chemical moderately (Table 1).

Table 1. Growth of Bacterial Isolates in Basal Medium Containing Acetaminophen as Carbon and Energy Source.

Rates of Bacterial Growth on Acetaminophen Coded Bacteria / Incubation Period (Days)	1	2	3	4	5	6
S. epidermidis AP03	+	+	+	+	+	+
Bacillus subtilis GU07	+	+ +	+ + +	+ + +	+ +	+ + +
Micrococcus sp. PS 10	+ +	+ +	+ +	+ +	'	+ +
Bacillus sp. PS 12	+	+	+ + +	+ + +	+++	+ + +
S. aureus PS 11		+	+	+	+	+
Streptococcus sp. AP0I						
Bacillus sp. AP02						
Bacillus cereus GU08						
Bsillus sp. GU09						
Micrococcus sp. CM04						
Bacillus sp. CM						
Streptococcus sp. CM06	—	—	—	—	—	—

+ + +: Maximum growth, + +: moderate growth, +: minimal growth, —: No growth.

Degradation of Acetaminophen by Bacterial Isolates

Micrococcus sp. PS 10 had the highest growth (Fig 1). The pH of its medium decreased most rapidly. The optical density of the medium increased sharply and its curve had the highest peak. The growth rate of ***Bacillus*** sp. PS12 was also high (Fig. 2). Its viable plate counts lagged for the first 5

days, peaked after 10 days and increased very gradually. The optical density also increased. ***Bacillus subtilis*** GU07 grew less rapidly with its viable plate counts reaching a peak after 25 days (Fig 3). The pH of its culture reduced from 7.2 to 6.0 after 25 days.

Discussion

The fact that only 3 out of the twelve isolates obtained were very active in degrading acetaminophen supports the assertion that most pharmaceutical products are aromatic.

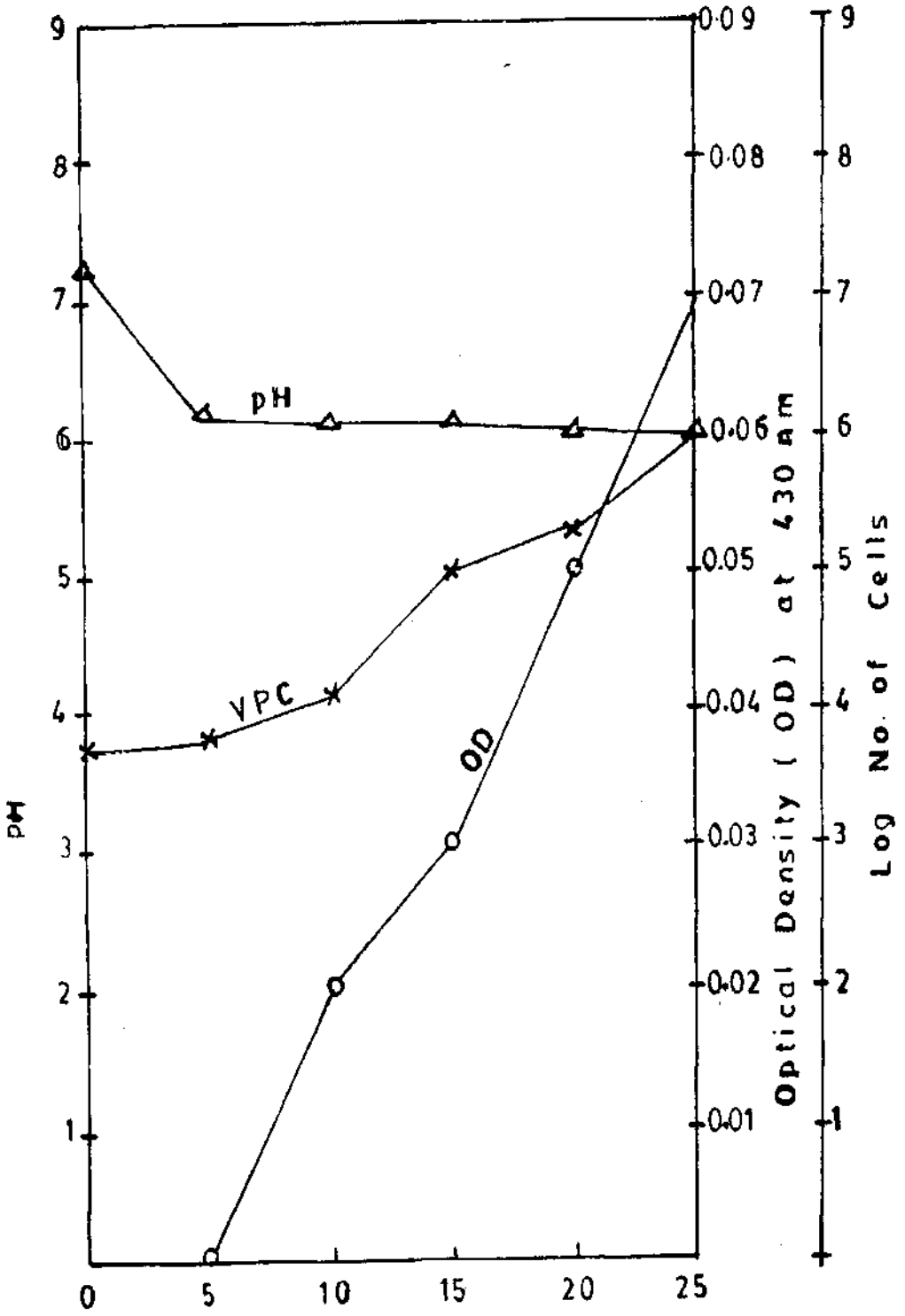

Fig. 1: Growth Profile of Micrococcus Sp. Ps10 in Mineral Salt Medium Containing Acetaminophen.

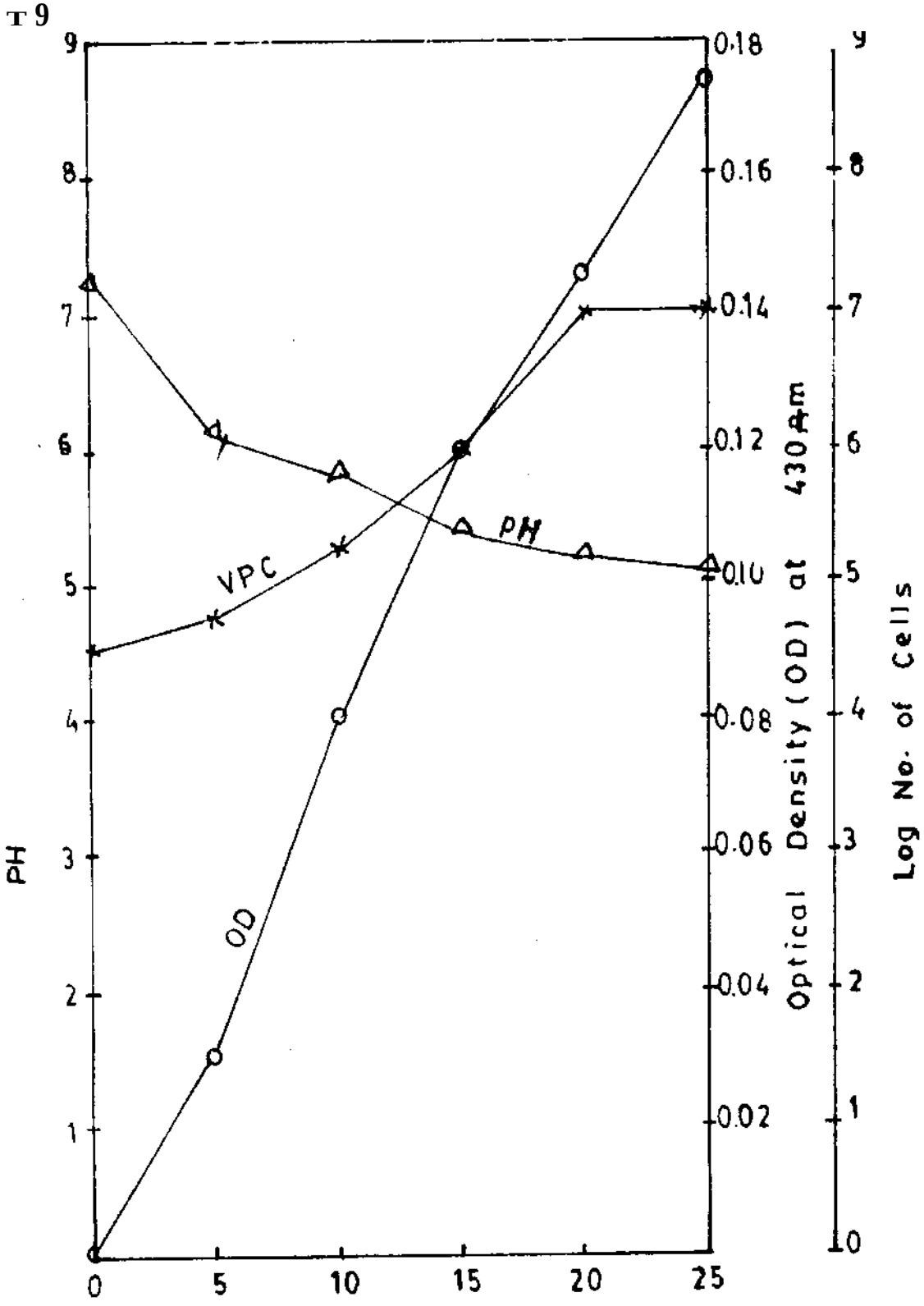

Fig. 2: Growth Profile of Bacillus Sp. Psl2 in Mineral Salt Medium Containing Acetaminophen.

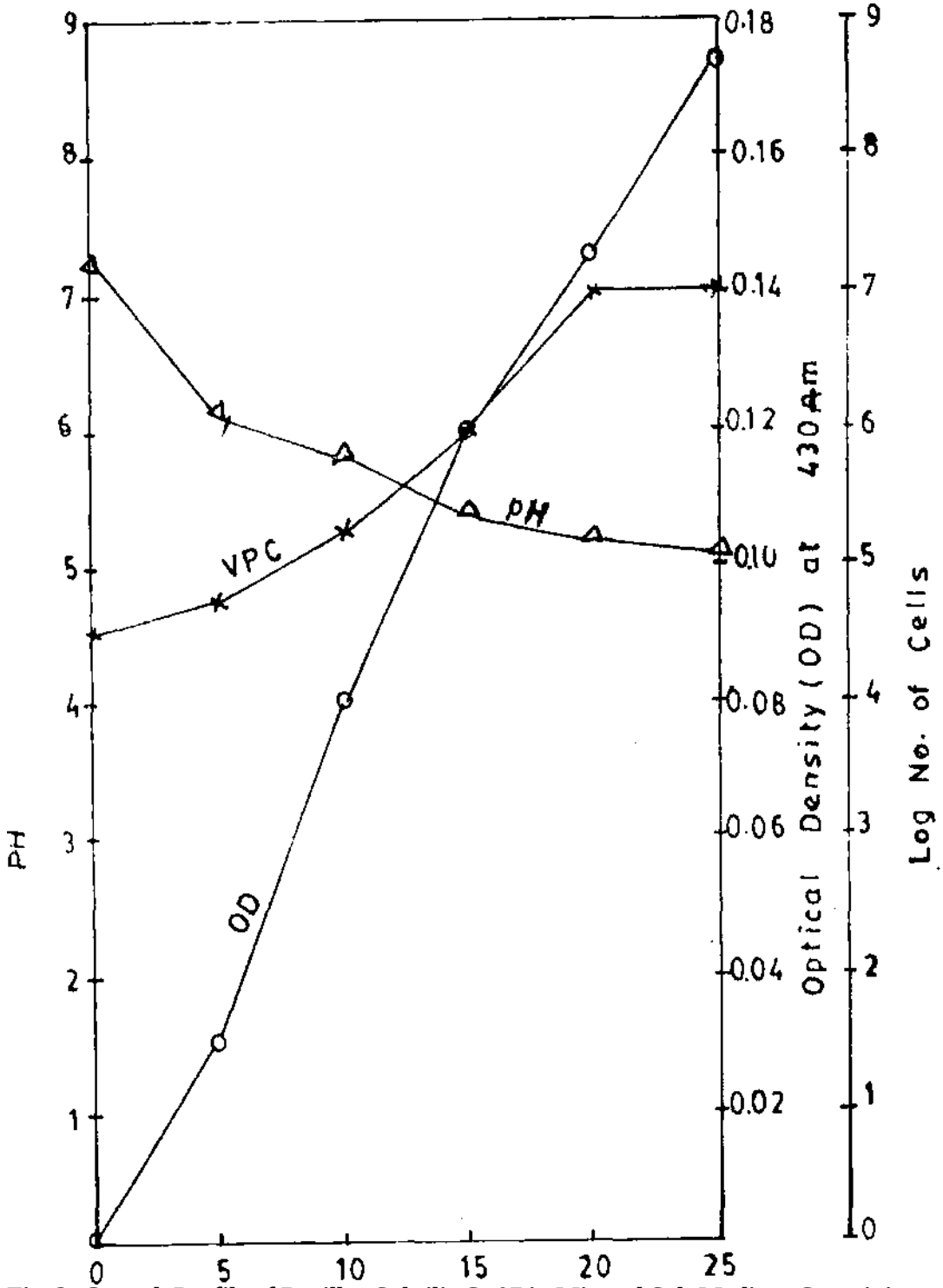

Fig. 2: Growth Profile of Bacillus Subtilis Gu07 in Mineral Salt Medium Containing Acetaminophen.

Molecules and are attacked by a smaller range of organisms. This is also reflected in the observation that ***Bacillus subtilis*** GU07 and ***Bacillus*** sp. PS 12 had considerable lag phases, 2 days for both during their growth on acetaminophen.

Micrococcus sp. PS 10 had the capacity to degrade acetaminophen as it practically had no lag phase compared with ***Bacillus subtillis*** GU07 and ***Bacillus*** sp. PS 12. This means that the organism had efficient degradative enzymes. The increase in turbidity is probably due to the fact that more breakdown products were present in the medium resulting in high turbidity. Low pH values in the medium indicate that a lot of acidic metabolites have been produced. The increase in viable counts showed that the incorporated acetaminophen has served as a source of carbon and energy for the organisms. The results of the present study are comparable to that of Eka ***et al.*** (1987) who found undesirable bacteria such as ***Bacillus subtillis*** and ***Streptococcus faecalis*** in non-sterile pharmaceutical preparations in Nigeria.

This study has established that the active component of paracetamol is prone to microbial breakdown. Paracetamol syrup as a formulation is more prone to contamination and spoilage since it contains many organic compounds that are readily utilizable by microorganisms. Though there are preservatives in the syrup, literature has it that even preservatives undergo degradation so that the presence of preservatives is not a guarantee to microbial safety. Therefore, the proliferation of contaminants in paracetamol syrups can lead to diseased conditions in consumers upon consumption if pathogenic species are present in large numbers. The most logical approach to minimizing the microbial hazard would be to specify that all products are manufactured as sterile products in single doze packs. Stringent laws on the manufacture and sales of syrups should be strictly enforced by the appropriate agencies.

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